IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Reissue Application No. 09/773,303   Group Art Unit: 1638

Filed: 31 January 2001   Examiner: Nelson, Amy J.

Reexamination of U.S. Patent No. 5,894,079   Confirmation No. 6243

For: FIELD BEAN CULTIVAR NAMED ENOLA

Applicant: Proctor, Larry M.   Date: 25 March 2003

BOX REISSUE
Commissioner for Patents
Washington, DC 20231

DECLARATION OF LAURA L. CONLEY
37 C.F.R. §1.132

1.   I have over five years of experience in the field of plant molecular biology, including research, development, and application of molecular markers to plant breeding. For more than two years I developed molecular markers and utilized molecular markers in plant breeding within the Biotechnology Department of Cargill Hybrid Seeds. I have a Master of Science degree in Biology from the California Institute of Technology and a Bachelor of Arts degree in Genetics from the University of California at Berkeley.

2.   This Declaration presents, for the Examiner's consideration, various facts which may be relevant to patentability of the claimed invention. These facts include an amplified fragment length polymorphism (AFLP) study showing genetic diversity among individual plants in Enola deposited with ATCC.

3.   Applicant has filed various Information Disclosure Statements, and among the Disclosure materials submitted is the Expert Report of Paul Gepts, Ph.D related to Civil Action No. 01-WY-2310-AJ (BNB). The Gepts Report is attached as Exhibit A to this Declaration. Dr. Gepts was used as an Expert Witness for an opposing party in litigation. Supportive data for Exhibit A is

attached as Exhibit B, and is also referred to herein as including pages DEF 001740 - DEF 001749.

4.   I have read Exhibits A and B.   I have also read the following article cited in the Report: P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper (1995) Nucl. Acids. Res. 23: 4407-4414 "AFLP: a new technique for DNA fingerprinting," which is attached as Exhibit C.

5.   Exhibit B includes supporting data for Exhibit A and was produced in litigaton discovery. The present Declaration is based on my analysis of Exhibit A, together with the supplemented raw data of Exhibit B.   Two separate experiments are described in the Gepts Report.   Exhibit B provides data from Experiments 1 and 2.   Within Exhibit B, DEF 001741 - DEF 001745 describe data from Experiment 1, and DEF 001746 - DEF 001749 describe data from Experiment 2.

6.   It is unclear from Exibit A alone, which lacks the data presented in Exhibit B, what methods are used to perform the described experiments; the description of the methods is incomplete and the methods as described are substantially different from the methods in the cited reference describing the AFLP technique (see Exhibit C as noted in paragraph 4 above).

7   The AFLP technique produces patterns of DNA fragments, which are visible as bands in an electrophoresis gel.   For an individual, an AFLP fragment may be present or absent.   A fragment that is present is typically scored as a "1," and a fragment that is absent is typically scored as a "0."   Data that is missing is typically scored as a "9."   When the patterns of fragments produced for two individuals are the same, the individuals are considered to have the same haplotype.   When the patterns of fragments produced for two individuals are different, the individuals are considered to have different haplotypes.

8.   AFLP fragments are typically tested on each individual in an experiment.   In Experiment 1, 151 AFLP fragments are scored, and in Experiment 2, 133 AFLP fragments are scored. Neither Exhibit A nor Exhibit B provides a cross-reference describing the relationship between any of the fragments scored in Experiments 1 and 2 or a rationale as to why fewer fragments are scored in Experiment 2 than in Experiment 1.

9.   Without a detailed description of the methods utilized to generate the data in Exhibits A and B, and without information addressing any relationship between the fragments scored in

Experiments 1 and 2, it is not possible to determine the cause of what appear to be inconsistencies between the results of Experiments 1 and 2.

10.   Exhibit A (Exhibit 2, Table 1 thereof) indicates that in Experiment 1, individual 1 is a first Enola 2000-1 seed from ATCC, and that individual 52 is a second Enola 2000-2 seed from ATCC.

11.   Exhibits A and pages DEF 001741 - DEF 001745 of Exhibit B describe data on 103 AFLP fragments (151 total - 48 fragments for which data is missing) for both individuals 1 and 52.

12.   Exhibit A and pages DEF 001741 - DEF 001745 of Exhibit B describe six AFLP fragments (3, 6, 20, 23, 36, and 86) that are present and/or absent in different patterns when comparing Enola individuals 1 and 52 (see Table 1 of the present Declaration).

13.   The results of these six AFLP fragments (3, 6, 20, 23, 36, and 86) for Enola individuals 1 and 52 demonstrate molecular heterogeneity in Enola deposited with ATCC. These results demonstrate that the haplotype of individual 1 is different from the haplotype of individual 52. These results demonstrate that there are at least two haplotypes within Enola deposited with ATCC.

14.   In Exhibit A (Exhibit 2, Table 1 thereof) and Exhibit B pages DEF 001741 - DEF 001745, individual 51 is Enola 2001 from Northern Feed and Bean (NF&B), and individual 53 (alternately designated B) is Enola-NFB from NF&B.

15.   Experiments in Exhibit A and Exhibit B pages DEF 001741 - DEF 001745 describe the analysis of 151 AFLP fragments, of which fourteen AFLP fragments (3, 6, 20, 23, 36, 50, 67, 68, 86, 110, 129, 131, 145, and 151, see Table 1 of the present Declaration) are present and/or absent in different patterns when comparing at least two of the Enola individuals 1, 51, 52, and 53.

Table 1[a, b]

AFLP fragment (Expt. 1)	1	52	51	53 (B)
	Enola 2000-1 ATCC	Enola 2000-2 ATCC	Enola 2001 NF&B	Enola-NFB NF&B
3	1	0	1	1
6	1	0	1	1
20	0	1	0	0
23	0	1	0	1
36	1	0	1	0
50	0	9	0	1
67	1	9	0	1
68	0	9	1	0
86	0	1	1	0
110	0	9	0	1
129	0	9	1	1
131	1	1	0	1
145	1	1	1	0
151	0	0	0	1

[a] A "1" indicates the presence of the fragment, a "0" indicates the absence of the fragment, and "9" indicates that no data is available.

[b] Table 1 is a subset of data presented in Experiment 1 of Exhibits A and B , as collated by myself.

16. The fourteen AFLP fragments described in Table 1 can be seen to be heterogeneous among Enola individuals 1, 51, 52, and 53. Furthermore, each of these four Enola individuals has a unique pattern of these fourteen AFLP fragments relative to each other. These data demonstrate the presence of at least four haplotypes within Enola from all sources.

17. Experiment 2 of Exhibit A describes a study of sixteen Enola individuals from ATCC (Exhibit A, page 18). Data from Experiment 2 (Exhibit B pages DEF 001746 - DEF 001749) is used to describe a single haplotype of Enola designated in Exhibit A as Haplotype A (Exhibit 8, Table 2 thereof).

18. It is also unclear from Exhibit A whether any of the sixteen Enola individuals tested in Experiment 2 were tested in Experiment 1. The labels used to identify samples in Experiment 2 (see Exhibit A page 18 and Exhibit B pages DEF 001746 - DEF 001749) match labels used for samples in Experiment 1 (Exhibit A, as shown in Exhibit 2, Table 1 thereof) for Mayacoba (#43 - 2001 sample of NF&B) and for Azufrado Peruano 87 (#49 - Azufrado Peruano 87 MPP), but

not for Enola (#1 - "sample of ATCC, acquired in 2002" in Exhibit A page 18 versus "Enola 2000-1" and "ATCC" in Exhibit 8, Table 2 of Exhibit A). The description of the Enola individuals in Experiment 2 (Exhibit A page 18, "sample of ATCC, acquired in 2002") is most similar to the description of individual 56 in Experiment 1 (Exhibit A as shown in Exhibit 2, Table 1 thereof, "Enola 2002" from "ATCC"). It is irrelevant, however, whether individual 56 in Experiment 1 is the same as one of or from the same source as the sixteen individuals tested in Experiment 2, because no data is presented for individual 56 in Exhibit A (for example, see Exhibits 4-6) or Exhibit B pages DEF 001741 - DEF 001745.

19.   Exhibit A does not provide a cross-reference of the fragments scored in Experiments 1 and 2, and Exhibit A does not provide data on any Enola individual common to both Experiments 1 and 2. Therefore it is not possible to determine if Haplotype A (as defined by Exhibit A) is or is not identical to one of the Enola haplotypes described in Experiment 1. Haplotype A may be an additional haplotype of Enola.

20.   Despite that Experiment 2 suggests that all Enola individuals from ATCC have the same haplotype (labeled Haplotype A in Experiment 2 of Exhibit A), Experiment 1 describes the presence of at least two haplotypes in Enola from ATCC (individuals 1 and 52).

21.   Exhibits A and B describe data for a total of eighteen (two in Experiment 1 and sixteen in Experiment 2) Enola individuals from ATCC. Data from Experiments 1 and 2, when combined, describe two or possibly three haplotypes in Enola deposited with ATCC. Experiments 1 and 2 describe data for a total of twenty (four in Experiment 1 and sixteen in Experiment 2) Enola individuals from all sources. The data in Experiments 1 and 2 suggest four or possibly five haplotypes within Enola from all sources combined.

22.   Exhibit A and Exhibit B pages DEF 001740 - DEF 001749 demonstrate that Enola is genetically diverse in that Enola contains at least four distinct haplotypes.

23.   The identification of a set of AFLP fragments that is identical for two individuals, does not "prove" homogeneity of those individuals. However, the identification of only one AFLP fragment that is different between two individuals does prove the heterogeneity of those individuals. Consequently, Experiment 2 (Exhibit A and Exhibit B pages DEF 001746 - DEF 001749) does not "prove" that the sixteen Enola individuals tested in Experiment 2 are identical or that Enola is homogeneous. The results of Experiment 2 only show that those sixteen Enola individuals are identical for the 133 markers tested in Experiment 2. The results of Experiment 1
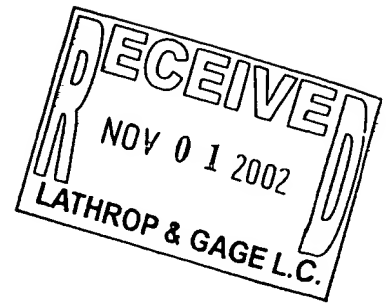
(Exhibit A and Exhibit B pages DEF 001741 - DEF 001745), however, do demonstrate AFLP fragments that score differently for Enola individuals (Table 1 of the present Declaration). The results of Experiment 1 do prove the heterogeneity of Enola, at least at the DNA sequence level. The results of Experiment 2 do not disprove the heterogeneity of Enola established by Experiment 1.

24.   I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Laura L Conley
Laura L. Conley M.S.

_3/25/03_
Date

IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF COLORADO

CIVIL ACTION NO. 01-WY-2310-AJ (BNB)

POD-NERS, LLC, a Colorado Ltd. Liability Co.)
)
Plaintiff,)
)
)
v.)
)
)
NORTHERN FEED & BEAN OF LUCERNE LTD.)
LIABILITY CO., a Colorado Ltd. Liability Co.; YELLOW)
RIVER, LLC, a Colorado Ltd. Liability Co.; HIGHLAND)
FEED & BEAN, INC., a Colorado Corp.; FLYING K)
FARMS, INC., a Colorado Corp.; WHITMAN FARMS,)
INC., a Colorado Corp.; MURATA FARMS, LLC, a)
Colorado Ltd. Liability Co.; LELAND BENSON, an)
Individual; LYNN FAGERBERG, an Individual;)
BARNARD GEISICK, an Individual; JIM GIBBS, an)
Individual; STAN HEINZE, an Individual; LOUIE)
FABRIZIOUS, an Individual; DENNIS KLEIN, an)
Individual; ROGER TROUDT, an Individual; CHUCK)
WINTER, an Individual; and STEVE WINTER, an)
Individual,)
)
)
Defendants.)
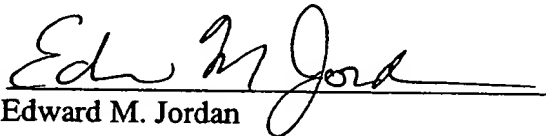
EXPERT REPORT OF PAUL GEPTS PH.D. IN SUPPORT OF MOTION FOR SUMMARY ADJUDICATION THAT PLAINTIFF'S PLANT VARIETY PROTECTION CERTIFICATE IS INVALID, OR IN THE ALTERNATIVE NOT INFRINGED

Attached hereto is a true and correct copy of the expert report of Paul Gepts, Ph.D, which was prepared in compliance with Fed.R.Civ.P. 26(a)(2)(B), and which is being filed in support of Defendants' motion for summary adjudication that Plaintiff's Plant Variety Protection certificate is invalid, or in the alternative is not infringed.

Respectfully submitted,

Jill M. Pietrini
Edward M. Jordan
MANATT, PHELPS & PHILLIPS, LLP
11355 West Olympic Boulevard
Los Angeles, California 90064
Telephone: (310) 312-4000
Facsimile: (310) 312-4224

Dated: October 31, 2002

By: _____
Edward M. Jordan

R. Sam Oldenburg
WITWER, OLDENBURG, BARRY & BEDINGFIELD, LLP
822 7th Street, Suite 760
Greeley, Colorado 80631
Telephone: (970) 352-3161
Facsimile: (303) 352-3165
Attorneys for Defendants
40557335.1

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF COLORADO

Civil Action No. 01-WY-2310-AJ (BNB)

POD-NERS, LLC, a Colorado Ltd. Liability Co.   )
)
       Plaintiff,   )
)
      v.   )
)
NORTHERN FEED & BEAN OF LUCERNE LTD.   )
LIABILITY CO., a Colorado Ltd. Liability Co.;   )
YELLOW RIVER, LLC, a Colorado Ltd. Liability Co.;   )
HIGHLAND FEED & BEAN, INC., a Colorado Corp.;   )
FLYING K FARMS, INC., a Colorado Corp.;   )
WHITMAN FARMS, INC., a Colorado Corp.;   )
MURATA FARMS, LLC, a Colorado Ltd. Liability Co.;   )
LELAND BENSON, an Individual;   )
LYNN FAGERBERG, an Individual;   )
BARNARD GEISICK, an Individual;   )
M GIBBS, an Individual;   )
STAN HEINZE, an Individual;   )
LOUIE FABRIZIOUS, an Individual;   )
DENNIS KLEIN, an Individual;   )
ROGER TROUDT, an Individual;   )
CHUCK WINTER, an Individual; and   )
STEVE WINTER, an Individual,   )
)
       Defendants.   )

EXPERT REPORT OF PAUL GEPTS, PH.D

I, Paul Gepts, have been retained by the attorneys for the defendants to provide expert testimony in this case. I submit this report pursuant to Rule 26(a)(2)(B) of the Federal Rules of Civil Procedure.

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A

A.   Experience

I am currently a full Professor of Agronomy in the Department of Agronomy and Range Science at the University of California, Davis. My responsibilities are twofold. In teaching, I have taught or I am teaching courses in general and plant genetics and in the evolution of crop plants (origins of agriculture). In the research area, I was hired to conduct research in crop biodiversity and its utilization in plant breeding..My research is focused particularly on beans (both *Phaseolus* and *Vigna* types) because California is one of the leading producing states of beans in the U.S.

I hold a degree of Ingénieur Agronome (specialization in Plant Protection) from the Faculté Universitaire des Sciences Agronomiques in Gembloux, Belgium (1976). This degree is equivalent to a M.S. from the U.S. I also hold a PhD in Plant Breeding and Plant Genetics from the University of Wisconsin, Madison, obtained under the guidance of Prof. F.A. Bliss (1984-1985). Subsequently, I did a postdoctoral training with Prof. M.T. Clegg at the University of California, Riverside in molecular evolution (1985-1987).

I have been involved in research and teaching of plant genetics and crop biodiversity, with particular emphasis on beans, for nearly 25 years. My career can be divided into three phases: a) Research associate from 1978 to 1981 in the Genetic Resources Unit of the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia; b) Research assistant and a postdoctoral fellow at the University of Wisconsin, Madison, and the University of California, Riverside (1981-1984 and 1985-1987, respectively); and 3) Faculty at the University of California, Davis, since 1987.

The Genetic Resources Unit at CIAT is responsible for the World Collection of

Phaseolus beans, including the common bean (*Phaseolus vulgaris*), which includes yellow-

seeded beans such as those under scrutiny in this action. During the three years I was active

there, I was involved in field evaluations of the genetic diversity for morphological and

agronomic traits of *Phaseolus* genetic resources. Because of the large size of the collection (more

than 30,000 entries), I became thoroughly familiar with the genetic diversity present in beans,

including wild beans and species related to common bean, for traits such as growth habit (plant

type: *e.g.,* climbing or pole beans vs. bush type), leaf type (shape, size, and color), phenology

(number of days to flowering and maturity), and seed types (color, color pattern, size, and

shape). I also evaluated progenies of crosses and became therefore familiar with genetic

segregations for these different traits and the effect of environmental effects on them (*e.g.,* year-

.ɔ-year variation).

A major contribution of my PhD thesis was to document the existence of two major

geographic gene pools and domestication centers (Andean and Mesoamerican) in common bean

using a biochemical marker, namely the electrophoretic variability of phaseolin, the major seed

storage protein of common bean. Most, if not all, common bean varieties are derived from one of

these two gene pools. This finding has had a major effect on 1) the conservation of genetic

diversity of common bean; and 2) on bean breeding because it helps bean breeders design better

crosses to develop improved bean varieties. One of my activities during my postdoctoral

fellowship was to edit a book devoted specifically to *Phaseolus* genetic resources, which was

published in 1988. Although now somewhat outdated, it is still a reference for individuals

interested in crop biodiversity, in particular for *Phaseolus* beans.

Since my appointment at UC Davis, I have risen quickly through the professorial ranks. I obtained tenure and was promoted from assistant to associate professor after only three years. I became full professor after another five years. I spent one year (1997-1998) on sabbatical leave at the Max-Planck Institute for Plant Breeding Research in Cologne, Germany, which is one of the leading plant molecular biology research centers in Europe. I have been invited twice to lecture at other universities, once in Argentina (Universidad de Rosario) and once in Italy (University of Perugia). From 1999 to 2001, I was chair of the department of Agronomy and Range Science at UC Davis.

Major research accomplishments during this period include:

a) Further documentation with molecular (RFLP, RAPD, AFLP) and biochemical (allozyme) markers of the existence of two major gene pools and domestication centers in common bean.

b) Identification within the Andean and Mesoamerican gene pools of six "races," each with distinct morphological and ecological adaptation traits.

c) Identification of the presumed ancestral wild populations of common bean in Ecuador and northern Peru.

d) Development of genetic linkage maps and localization on these maps of genes for traits pertaining to the domestication syndrome, disease resistance, and tolerance to insects.

e) Genomic analysis of the evolution of a small multigene family involved in seed weevil resistance.

As a consequence, I am now recognized nationally and internationally as one of the leading experts in crop biodiversity and domestication, particularly that of common bean. My expertise ranges from field studies (both field explorations in Latin America and field trials in a variety of locations) to detailed molecular (DNA) analyses of genetic diversity. My work has been recognized recently by election as a fellow of the American Association for the Advancement of Science and as a board member of the American Genetics Association. Furthermore, I have been actively engaged in activities related to bean genetic resources. I was chair of the Genetics Committee of the Bean Improvement Cooperative, a voluntary organization of bean researchers from around the world. I am still a member of that committee. I was also chair of the USDA Phaseolus Crop Germplasm Committee and I continue to be a member of this committee, which discusses issues related the maintenance, evaluation, and acquisition of bean germplasm for the USDA bean collection.

My curriculum vitae is attached hereto as Exhibit 1.

B.   Expert In This Case

At the request of defendants' attorneys, I have been retained to determine the genetic relationships at the molecular level between the cultivar 'Enola' and a yellow-seeded cultivar grown by the defendants, named 'Mayocoba.' Such an analysis also requires examining molecular relationships between the above-named cultivars and other bean cultivars, mainly yellow-seeded cultivars from Mexico, but other cultivars as well in order to: 1) verify that the AFLP technique detects similar patterns of genetic diversity as other molecular or biochemical markers based on our earlier experience; 2) determine the value of AFLP markers to distinguish

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closely related cultivars of common bean; and 3) determine the range of genetic distances or similarities prevailing in common bean, *Phaseolus vulgaris*, the species to which Enola and similar cultivars belong.

Two main experiments were conducted. **A first experiment** was set up to determine the genetic relationships at the molecular (DNA) level among yellow-seeded cultivars and between yellow-seeded cultivars, on the one hand, and other bean cultivars, on the other hand. The main goal was to determine whether AFLP markers detect the same genetic relationships as other markers used previously in our analyses of genetic diversity of beans. An additional goal was to determine whether cultivars ("cvs.") Enola (Podners) and Mayocoba (Northern Feed and Bean; NF&B) were the same or distinct materials. This required analyzing not only those cultivars but also other cultivars to determine whether the observed genetic distance between Enola and Mayocoba is biologically significant within the species. I define a *marker* here as an easily scorable trait (*i.e.*, present or absent) with a simple inheritance (*i.e.*, a single gene). Originally, the term marker was used for morphological traits satisfying this definition. For example, this could be flower color (*e.g.*, white vs. purple) in plants or eye color in humans. More recently, this term has been extended to DNA molecules as well, where a marker designates a specific DNA sequence (for further explanations about DNA, see below). Markers are important genetic tools because they can be scored unambiguously in every individual as present or absent and they correspond to a single location in an organism's *genome* (defined as the sum of its heritable material).

For this first experiment, a sample of 56 bean cultivars was assembled from several sources (Table 1, attached hereto as Exhibit 2). Twenty-four of these cultivars have yellow seeds.

Some of the samples may represent the same cultivar but were obtained from different sources (*e.g.*, 46 & 49, 47 & 48 in Table 1) or were grown or obtained in different years (*e.g.*, 42 & 43, 1 & 56 in Table 1). They were nevertheless all included in the analysis. There were four main sources of seed materials:

a) the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, for entries representative of bean (*Phaseolus vulgaris*) genetic resources in general and some yellow-seeded breeding lines from Mexico. CIAT is the repository for the World Collection of Phaseolus, *i.e.*, it is the reference collection of bean cultivars. In particular, this sample from CIAT included representatives of the six major domesticated races (Table 1),[1] which assured us that the sample was broadly representative of the genetic diversity of bean cultivars in Latin America.

b) the American Type Culture Collection (ATCC), which houses the standard sample of the Enola cultivar.

c) Manatt, Phelps, and Phillips (MPP): samples of Mexican varieties belonging to the Peruano commercial class: Azufrado Pimono 78, Azufrado Peruano 87, and Azufrado Regional 87.

d) Northern Feed and Bean (NF&B): samples of their yellow-seeded cultivar called 'Mayocoba,' as well as cvs. 'Enola' and 'Myasi.'

Additional seed samples were provided by S. Temple (UC Davis: Canario 707, an experimental yellow-seeded line), J. Acosta (INIFAP: two of the same lines provided by Manatt, Phelps, and Phillips: Azufrado Peruano 87 and Azufrado Regional 87), and J. Nienhuis and K.

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[1] Singh SP, Gepts P, Debouck DG (1991) Races of common bean (*Phaseolus vulgaris* L., Fabaceae). *Economic Botany* **45**, 379-396.

Kmiecik (University of Wisconsin, Madison: 'Sulphur,' a U.S. traditional cultivar with yellow seeds, already described by Hedrick in 1931[2] and obtained from the Seed Savers Exchange, Decorah, IL). In addition, two samples of yellow-seeded beans were purchased in stores, one in Woodland, CA ("Woodland Yellow"), and the other in Seattle, WA ("Frijol Canario"). The latter was advertised on the packaging as an authentic Peruvian yellow bean.

In this first experiment, one individual seed was analyzed for each entry because initially it was more important to determine overall levels of genetic relatedness among different cultivars. Our prior experience in this area showed that most of the genetic differences reside among cultivars and much less within cultivars, an observation consistent with the self-pollinating nature of common bean.

The seeds were germinated in a greenhouse under uniform conditions. Plants were fertilized regularly and kept free of diseases and especially pests. DNA was extracted from leaf tissue harvested when plants had anywhere between two and five leaves, i.e., before flowering. The DNA extraction method was as described by Gepts and Clegg (1989),[3] but without the addition of polyvinylpolypyrrolidone (PVPP). Genetic relationships were assessed using the Amplified Fragment Length Polymorphism (AFLP) technique (Vos et al. 1995).[4] This technique is a very powerful means of establishing the degree of genetic relatedness among different individuals (even those that cannot readily be distinguished by morphological or biochemical means) because it simultaneously surveys a large number of markers (i.e., DNA sequences)

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[2] Hedrick U (1931) *The vegetables of New York. I. Legumes, curcurbits, corn, alliums, asparagus. Part II. Beans* J.B. Lyon, Albany, NY.
[3] Gepts P, Clegg MT (1989) Genetic diversity in pearl millet (*Pennisetum glaucum* [L.] R.Br.) at the DNA sequence level. *Journal of Heredity* **80**, 203-208.

distributed throughout the genome of individuals. It also provides very reproducible results in comparison to other types of markers.

The basic principle of the AFLP technique is as follows. DNA is the biochemical vehicle of hereditary information in living organisms. It specifies the morphological, physiological, and behavioral characteristics of an organism, *i.e.*, how an organism looks and functions. Because DNA is transmitted from parents to the progeny, children have a tendency to resemble their mother and father and even earlier generations. The DNA molecular itself consists of a double helix. In turn, each helix consists of a chain of four different building blocks, named A, C, G, and T (abbreviations of their chemical names). The specific order (*i.e.*, the sequence) of these building blocks is important as it ultimately determines the characteristics of any organism. Different sequences can result in morphological and other differences among individuals. How does the AFLP technique then detect differences in the DNA sequence of different individuals?
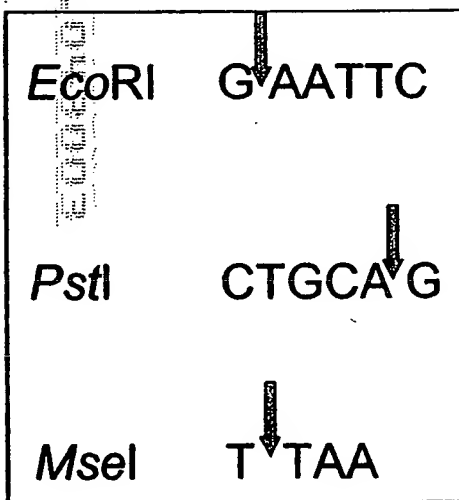
The technique uses enzymes that cleave the DNA at specific spots determined by the actual DNA sequence itself. For example, the enzymes most often used in this technique –

*Eco*RI, *Pst*I, and *Mse*I – cut at the DNA sequences shown to the left (with the arrow indicating the specific location of the cut). Whenever any of these sequences are present they will be cut by the enzymes. If there is a change in the

*Eco*RI	G▼AATTC
*Pst*I	CTGCA▼G
*Mse*I	T▼TAA

[4] Vos P, Hogers R, Bleeker M, *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

sequence, for example, if GAATTC becomes GGATTC, then the DNA will not be cut at that location.

A longer sequence in two genetically related but distinct individuals is shown below:

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Individual A

... ACTTCTTTCG AATTCATCCATCG AATTCCAGAGTACTACTACTCTACTACTT TAATACCCCAACC


Individual B

... ACTTCTTTCG AATTCATCCATCG GATTCCAGAGTACTACTACTCTACTACTT TAATACCCCAACC
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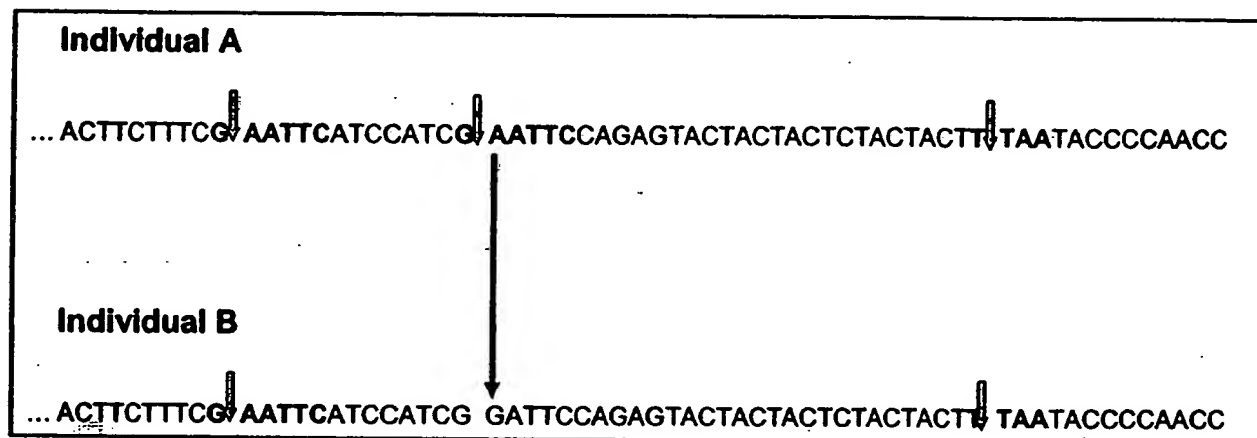
Individual A has – in the sequence shown here – two sites (GAATTC, marked in bold) where the enzyme EcoRI can cleave the DNA molecule and one site (TTAA, also marked in bold) where the enzyme MseI can cut DNA. In individual B, a change in one of the building blocks, from A to G (indicated by the black arrow) eliminates one of the cutting sites. Thus, individual B will show only one large fragment in this DNA region. The size of this fragment is exactly the sum of the two smaller fragment shown by individual A. To visualize these differences in the number and sizes of fragments, the enzyme-treated DNA is subjected to electrophoresis, a technique that separates molecules according to size.

Figure 1 (attached hereto as Exhibit 3) shows an example of the result of such an electrophoresis. Each vertical lane (B1, B2, 1 through 34) is a different bean line. Each line has a characteristic pattern of bands (Fig. 1). Some bands are present in some bean lines, whereas others are missing. Using the presence/absence pattern of individual bands, one can thus

listinguish different bean lines. (The dots on the figure are manual additions to facilitate the scoring of individual bands.)

The presence of a band is scored as a 1, whereas its absence of scored as a 0. When all bands are scored, one ends up for each cultivar with a suite of ones and zeroes resembling a bar code. Individual A could have the following suite:

11100100010011

whereas Individual B could have the following suite:

11000011010001

(with the numbers in bold representing a difference with individual A). This suite is characteristic of each individual and constitutes its so-called *haplotype*. Thus, a haplotype of an individual is a specific combination of the presence and absence of DNA sequences sampled from that individual's genome. Just like a bar code in a grocery store is unique for each item sold in the store, the haplotype refers to an individual with a particular genetic constitution and not any other individual with a different genetic constitution. In the case of the first experiment, our bar codes or haplotypes included 151 ones and zeroes. In the second experiment, our haplotypes included 133 ones and zeroes. In both cases, these are very large haplotypes, a reflection of the high level of detail of our experiments. The larger the number of markers (ones and zeroes) the higher the possibility of identifying differences among cultivars. Conversely, if two individuals are alike for such a large number of sequences, they are very similar indeed.

In order to facilitate the representation of these results, additional calculations are performed to simplify the overall results. Indeed, each fragment or band in the gel shown below is considered a variable or dimension. With over 100 fragments to analyze for each bean line,

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there would also be over 100 dimensions. It is very difficult if not impossible for the human

mind to visualize the results in a space of over 100 dimensions. Therefore, the calculation

methods reduce this large number of variables down to one to at most three variables, which are

composite variables. Two methods were used here to achieve this objective. A first method was a

clustering or grouping method using a calculation method called the Unweighted Paired Group

Method using Arithmetic Averages or UPGMA clustering (giving rise to a *dendrogram* or tree-

like drawing showing genetic relationships in one dimension). I define a *cluster* here as a group

of closely related cultivars. The UPGMA method identifies successively larger groups or clusters

of related cultivars based on average genetic similarities among cultivars. The second method

was a Principal Coordinate Analysis (giving rise to a three-dimensional drawing showing the

genetic relationships among cultivars), as implemented by the software NTSYS (Rohlf 1997).[5]

More specific explanations about these methods will be given below when results are presented.

In the **second experiment**, three yellow-seeded bean cultivars were analyzed to

determine the variability within each cultivats. The three cultivars were: 1) Enola obtained from

ATCC, sample of 2002; 2) Mayocoba obtained from NF&B; sample of 2001; and 3) Azufrado

Peruano 87, obtained from Manatt, Phelps, and Phillips. For each of these varieties, an additional

15 individuals were analyzed for a total of 16 per cultivar (including the single individual

analyzed in the first experiment). The molecular analysis methods were the same as those

described for the first experiment. Results were also analyzed by UPGMA clustering and

Principal Coordinate Analysis.

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[5] Rohlf FJ (1997) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.02. Exeter Software, Setauket, New York.

Furthermore, the statistical significance of the major clusters or groups of cultivars in the dendrogram of the second experiment (see below) was assessed by bootstrapping[6] the data as implemented in the software program WINBOOT (http://www.irri.org/winboot.htm) . This statistical analysis allows us to determine whether the dendrogram is significantly different from one that could be obtained at random. The program calculates a value (the so-called *bootstrap value*) ranging between 0 and 100, which expresses how consistently entries fall into the cluster after random resampling (with replacement). To determine this, the existing data points for each individual are resampled with replacement and a new dendrogram is constructed. *Resampling means* that each data point for a cultivar (*i.e.*, a 0 or 1 for a given band observed by electrophoresis) is replaced by another data point obtained by sampling among the existing data points (*i.e.*, 0s or 1s for the same band among all cultivars).

Following each resampling, a new dendrogram is calculated. If some cultivars continue to appear in the same cluster in the dendrogram after the resampling, that is taken as a sign that the cultivars indeed belong to this same cluster. This operation of resampling, clustering, and dendrogram building is repeated a large number of times (here 10,000 times, which is larger than what is usually done) using a computer. The analysis then counts how often entries appear in the same cluster in all of the 10,000 analyses. This frequency, expressed in percentage, is the bootstrap value mentioned before. It ranges between 1 and 100. The higher the value, the stronger the statistical support for the value. Thus, a value of 100 tells us that in 100 out of 100 trials the entries fall into that same cluster. This value is attained when all entries have the same haplotype. Progressively lower values are achieved when entries are less consistently grouped in

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[6] Efron B (1979) Bootstrap methods: another look at the jackknife. *Annals of Statistics* 7, 1-26.

the same cluster. In practice, a minimum cutoff value of 50 is chosen above which clusters are accepted as statistically valid and below which clusters are considered to be unreliable. The higher the values are above 50, the higher the degree of confidence in the cluster. In this experiment, the bootstrap values were determined after 10,000 trials or resamplings, well above the usual number of repetitions usually performed for this type of assay.

Both experiments were performed under my supervision in my laboratory at the University of California, Davis. The personnel involved in the analyses is highly proficient in the AFLP techniques and the statistical analysis of their results. However, they are not familiar with bean genetic diversity *per se*. In addition, we used consecutive numbers and letters (see leftmost and fourth column in Table 1) to avoid direct knowledge of the materials analyzed until the interpretation phase at the end of the experiments. Thus, these experiments can be considered blind tests, which reduce or eliminate any inadvertent bias in the experimental design and data reading.

C.   Expert Opinion And Basis Therefore

For the reasons stated in more detail below, it is my opinion that the sample of the cultivar Mayocoba, as provided to me by Northern Feed and Bean (NF&B), is distinct at the molecular level from the sample of the cultivar Enola obtained from the American Type Culture Collection. It includes several combinations of molecular sequences (the so-called haplotypes) not present in Enola. In contrast, the single haplotype detected in cv. Enola is not unique, but also appears in Mayocoba and Azufrado Peruano 87, the latter of which pre-dates the development of Enola. Molecular data, therefore, show that Enola is not a novel nor unique bean

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)genotype as I will explain in the following paragraphs and with the help of several figures and a

table. Our observations complement data of Bassett et al. (2002)[7] demonstrating that the yellow

seed color in Enola is controlled by the same gene combination as in other, older yellow-seeded

cultivars as I will explain later also.

· The results of the first experiment show that the Peruano commercial class to which

Enola belongs is a tight-knit subgroup of the Andean gene pool of common bean (Fig. 2,

attached hereto as Exhibit 4) as explained in this paragraph. As Fig. 2 illustrates, in confirmation

of previous results, there are two types of common bean. The group to the left of the figure

constitutes the Mesoamerican group, in which the cultivars are derived from bean plants that

were domesticated in Mexico. The group to the right, which includes Enola, represents the

Andean gene pool in which cultivars are derived from plants domesticated in the southern half of

the Andes (Gepts 1993, 1998).[8]

Peruano cultivars initially resulted from crosses performed by Mexican bean breeders,

starting with Hector López in the 1960-70s, between a cultivar from the Mesoamerican gene

pool with cream-to-yellow seeds and colored corona rings (belonging to the group at the left in

Fig. 2) and a cultivar from the Andean gene pool with more intensely yellow seeds but light

corona and hilum rings (belonging to the group at the right in Fig. 2), as documented by Voysest

[7] Bassett M, Lee R, Otto C, McClean P (2002) Classical and molecular genetic studies of the strong greenish yellow seedcoat color in 'Wagenaar' and 'Enola' common bean. *Journal of the American Society for Horticultural Science* 127, 50-55.

[8] Gepts P (1993) The use of molecular and biochemical markers in crop evolution studies. *Evolutionary Biology* 27, 51-94.
Gepts P (1998) Origin and evolution of common bean: past events and recent trends. *HortScience* 33, 1124-1130.

) (2000).[9] A representative cultivar similar to the Mesoamerican parent was included in our sample

(Mayocoba: see Fig. 2, left group) as was a representative of a Peruvian Canario cultivar (Frijol

Canario: see Fig. 2, right group).

The location of the Peruano group within the Andean gene pool instead of in an

intermediate position between the Andean and Mesoamerican gene pools can be explained by a

breeder's selection for plant and seed characteristics of the Andean parent. Genes for these traits

are spread over the entire bean genome (Gepts 1999, Bassett et al. 2002, McClean et al. 2002).[10]

Thus, selection for the Andean phenotypes controlled by these genes will lead to a genome that

is predominantly Andean in nature. Of interest is also that the heirloom cultivar Sulphur with

yellow seeds (described by Hedrick in 1931)[11] belongs to the Andean group although it is located

at its margin (Fig. 2). This particular location reflects a hybrid origin as both its seed size and its

main seed protein reflect a Mesoamerican contribution (Gepts et al. 1988).[12]

A different view of the results of the first experiment is provided by a dendrogram (Fig.

3, attached hereto as Exhibit 5). A dendrogram is a tree-like drawing showing the genetic

similarity among cultivars along a single axis shown at the bottom of the drawing (Fig. 3). In our

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[9] Voysest O (2000) *Mejoramiento genético del frijol: legado de variedades de América Latina 1930-1999* Centro Internacional de Agricultura Tropical, Cali, Colombia.
[10] Gepts P (1999) Development of an integrated genetic linkage map in common bean (*Phaseolus vulgaris* L.) and its use. In: *Bean breeding for the 21st century* (ed. Singh S), pp. 53-91, 389-400. Kluwer, Dordrecht, the Netherlands.
Bassett M, Lee R, Otto C, McClean P (2002) Classical and molecular genetic studies of the strong greenish yellow seedcoat color in 'Wagenaar' and 'Enola' common bean. *Journal of the American Society for Horticultural Science* 127, 50-55.
McClean P, Lee R, Otto C, Gepts P, Bassett M (2002) Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). *J Hered* 93, 148-152.
[11] Hedrick U (1931) *The vegetables of New York. I. Legumes, curcurbits, corn, alliums, asparagus. Part II. Beans* J.B. Lyon, Albany, NY.

analyses, we have used Dice's genetic similarity, which is commonly used in studies of genetic

relationships among living organisms. Dice's similarity between two groups is defined as

$$D = \frac{2a}{2a + b + c},$$

where a is the number of bands observed in a gel such as shown in Fig. 1 shared by the two

groups, b is the number of bands present in one group but absent in the other, and conversely for

c. If all bands are shared by the two groups (*i.e.*, they are identical), then D = 1. In contrast, when

no bands are shared (*i.e.*, they are completely different), then D= 0.

The average similarity between clusters of cultivars can be read by prolonging the

vertical bar linking these two groups in the dendrogram down to the horizontal axis of Dice's

genetic similarities. For example, in Fig. 3, the large cluster at the top of the dendrogram, which

includes some two thirds of the cultivars assayed, has a similarity of only 0.42 to the lower,

smaller cluster. Fig. 3 also reveals the cultivars most closely related to Enola (at an average

similarity of 0.98). These include a U.S. cultivar, Myasi 2001 (provided by NF&B; also labeled

ADM in an earlier sample from NF&B), Azufrado Peruano 87 (provided by MPP), and

"Woodland Yellow" (bought in a grocery store in Woodland, CA, in 2000). The vertical bar

uniting Myasi 2001 and ADM at a Dice similarity of 1.0 shows these samples had the same

haplotype, *i.e.*, they were identical..

Fig. 4 (attached hereto as Exhibit 6) is a close-up of the Peruano group shown in Fig. 2.

The distance between circles reflect the genetic distances between the different cultivars

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[12] Gepts P, Kmiecik K, Pereira P, Bliss FA (1988) Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. *Economic Botany* 42, 73-85.

included in the experiment. The main observation of this Figure is that different individuals of the same cultivar from the same source generally show small differences at the molecular level as revealed by a slightly different position in the graph. This holds in particular for the following varieties (surrounded by a blue ellipse in the Figure): a) Azufrado Peruano 87; b) Azufrado Regional 87; c) Enola (NF&B); and d) Mayocoba (NF&B). These molecular differences raise the issue of uniformity of the different cultivars.

A second experiment was therefore conducted specifically to assess the level of molecular diversity in a sample of three Peruano-type cultivars: Enola (sample of ATCC acquired in 2002), Mayocoba (2001 sample of NF&B), and Azufrado Peruano 87 (MPP). The dendrogram resulting from this experiment (Fig. 5, attached hereto as Exhibit 7) reveals two major differences with the dendrogram from the first experiment. Firstly, the materials are – expectedly – much more similar as shown by the higher similarity (0.935) between the two most different clusters. Secondly, there were several groups of individuals that showed the same haplotype. Individuals within these groups are united in the dendrogram by a vertical line corresponding to a genetic similarity of 1.0. For example in Fig. 5, all the Enola individuals tested (in red color), as well as five Azufrado Peruano 87 (in green) and one Mayocoba individual (in blue) showed the same haplotype (top half of the dendrogram). Furthermore, the three cultivars displayed different haplotypes (arrays of molecular alleles). Table 2 (hereto attached as Exhibit 8) lists the 12 different haplotypes observed, labeled A through L. The three cultivars could be distinguished both qualitatively (which haplotypes?) and quantitatively (how many haplotypes?). The most diverse sample was that of Azufrado Peruano (provided by MPP), which included eight different haplotypes (A, F-L). The second most diverse sample was that of

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Mayocoba 2001 provided by NF&B with five haplotypes (A-E). In contrast, the Enola sample (from ATCC) was uniform with a single haplotype (A). Furthermore, this haplotype A found in Enola was also found in the two other cultivars. Therefore, haplotype A, the molecular signature of Enola, pre-existed among the different haplotypes characterizing the Mexican cultivar Azufrado Peruano 87, which is known to have preceded Enola.

The statistical significance of the difference in distribution of the different haplotypes is confirmed by a bootstrap analysis. Fig. 5 shows that Mayocoba is very distinct from the two other cultivars assayed in that the bootstrap value for the cluster including most of the Mayocoba haplotypes is very high (96 out of a maximum of 100). The cluster containing the Enola and Azufrado Peruano 87 representatives has a moderately high bootstrap value (67 out of 100), strongly suggesting they belong in the same cluster. This is further confirmed by the low bootstrap value for the Azufrado Peruano 87 cluster (42). The latter observation shows that Enola in our test is not distinct from Azufrado Peruano 87.

I conclude that, at the molecular level, Mayocoba is distinct from Enola because it contains several haplotypes not included in Enola. Enola, in contrast, is not a unique or novel cultivar as it contains a single haplotype that pre-existed in the yellow-seeded cultivar Azufrado Peruano 87. Our observations at the molecular level are consistent with those made by Bassett et al. (2002)[13] on the identity of genes controlling seed color in Enola. Bassett et al. (2002) were able to show that the genes controlling the yellow seed color in Enola were the same as those in

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[13] Bassett M, Lee R, Otto C, McClean P (2002) Classical and molecular genetic studies of the strong greenish yellow seedcoat color in 'Wagenaar' and 'Enola' common bean. *Journal of the American Society for Horticultural Science* **127**, 50-55.

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the old Dutch cultivar Wagenaar analyzed previously by Prakken (1970, 1972).[14] Thus, neither at

the molecular level nor for the seed color trait is Enola a novelty or a distinct cultivar.

My opinions set forth above are based upon:

a) Information about the history and geographic distribution of yellow-seeded varieties,

   particularly the Peruano class, as documented by Hedrick (1931), Dorschne (1992),

   and Voysest (2000).[15]

b) Information from the analyses of yellow-seeded cultivars (Sulphur, Azufrado Pimono

   78) as published in my PhD thesis (Gepts 1984)[16] and articles or chapters derived

   from them (Gepts et al. 1986; Gepts et al. 1988; Gepts 1988).[17]

c) Information from phenotypic and biochemical analyses of genetic diversity in the

   domesticated gene pool of common bean as published in Singh et al. (1991a,b,c).[18]

[14] Prakken R (1970) Inheritance of colour in *Phaseolus vulgaris* L. II. Critical review. Mededelingen Landbouwhogeschool Wageningen 72-23, 1-38.
Prakken R (1972) Inheritance of colour in *Phaseolus vulgaris* L. III. On genes for red seedcoat colour and a general synthesis. Genetics 72-29, 1-82.
[15] Hedrick U (1931) *The vegetables of New York. I. Legumes, curcurbits, corn, alliums, asparagus. Part II. Beans* J.B. Lyon, Albany, NY.
Dorschne C (1992) Horticultural has-beans. In: *National Gardening*, pp. pp. 34-37.
Voysest O (2000) *Mejoramiento genético del frijol: legado de variedades de América Latina 1930-1999* Centro Internacional de Agricultura Tropical, Cali, Colombia.
[16] Gepts P (1984) *Nutritional and evolutionary implications of phaseolin seed protein variability in common bean (Phaseolus vulgaris L.)* Ph.D. dissertation, University of Wisconsin, Madison.
[17] Gepts P, Osborn TC, Rashka K, Bliss FA (1986) Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Economic Botany* 40, 451-468.
Gepts P, Kmiecik K, Pereira P, Bliss FA (1988) Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. *Economic Botany* 42, 73-85.
Gepts P (1988) Phaseolin as an evolutionary marker. In: *Genetic resources of {IPhaseolus} beans* (ed. Gepts P), pp. 215-241. Kluwer, Dordrecht, the Netherlands.

d) Research conducted recently in my laboratory on genetic diversity of yellow-seeded

beans and presented here.

The exhibits that I will use to testify as a summary of, or in support for, my opinion are:

a) This report, including its figures and tables.

D.   Compensation

I am being compensated for my services as an expert witness in this matter at a rate of

$400 per hour. My compensation is not contingent upon the outcome of the trial or upon the

nature of my opinions.

E.   Publications In The Last Ten Years

Within the last ten years, I have authored the publications listed in my CV, attached

hereto as Exhibit 1.

F.   Cases Where Testified As An Expert In The Last Four Years

I have not testified as an expert witness at trial or by deposition in any other case within

the preceding four years.

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[18] Singh SP, Debouck DG, Gepts P (1989) Races of common bean, *Phaseolus vulgaris* L. In: *Current topics in breeding of common bean, Proc. Intern. Bean Breed. Workshop* (ed. Beebe S), pp. 75-89. CIAT, Cali, Colombia.
Singh SP, Gutiérrez JA, Molina A, Urrea C, Gepts P (1991) Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. *Crop Science* **31**, 23-29.
Singh SP, Nodari R, Gepts P (1991) Genetic diversity in cultivated common bean. I. Allozymes. *Crop Science* **31**, 19-23.

Executed this 5th day of October 2002 at Davis, California.

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Paul Gepts, Ph.D.

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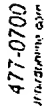
Exhibit 1

CURRICULUM VITAE

Paul Gepts

A. PERSONAL DATA:

Office address : Dept. of Agronomy and Range Science
University of California
Davis, CA 95616-8515
Phone: (530) 752-7743 or (530) 752-2723
FAX: (530) 752-4361
E-mail: plgepts@ucdavis.edu
WWW: http://agronomy.ucdavis.edu/gepts/geptslab.htm

Nationality: Belgian
Languages: French, Flemish (Dutch), English, Spanish (all fluently spoken and written),

B. EDUCATION:

1971-73 : Faculté des Sciences Agronomiques, Gembloux, Belgium
Candidature en Sciences Agronomiques

1973-76 : Faculté des Sciences Agronomiques, Gembloux, Belgium
Ingénieur agronome, orientation Défense des Végétaux. Advisor:
Dr. J. Semal.
Thesis: "In vitro synthesis of double-stranded Tymovirus RNA:
specific dilutions of the labeled products by homologous
and heterologous RNAs".

1981-84 : University of Wisconsin, Madison, Wisconsin, USA
Ph.D.: Major: Plant Breeding and Genetics under Dr. F.A. Bliss;
Minor: Botany
Thesis: "Nutritional and evolutionary implications of phaseolin
seed protein variability in common bean (*Phaseolus
vulgaris* L.)."

C. ADDITIONAL TRAINING:

1976 (6 months) : Faculté des Sciences Agronomiques, Gembloux, Belgium
Tropical agriculture

1978 (1 month) : Station des Cultures fruitières et maraîchères, Gembloux, Belgium
Tissue culture

D. PROFESSIONAL RECORD:

1977-78 : Faculté des Sciences Agronomiques, Gembloux, Belgium
Research assistant
Chemical control of cereal diseases

1978-81 : Centro Internacional de Agricultura Tropical, Cali, Colombia
Research associate
Interspecific hybridizations in common bean breeding

1981-84 : University of Wisconsin, Madison, Wisconsin, USA
Research assistant
Seed proteins of the common bean: nutritional and evolutionary
aspects

1985-87 : University of California, Riverside, California, USA
Visiting postdoctoral research geneticist
Genetic variability of nuclear and chloroplast DNA sequences in
pearl millet

1987-90 : University of California, Davis, California, USA
Assistant Professor
Genetics of food legumes

1990-95 : University of California, Davis, California, USA
Associate Professor
Genetics of food legumes

1995-present : University of California, Davis, California, USA
Professor
Genetics of food legumes

1997 (March) : Universidad Nacional de Rosario, Argentina
Visiting Professor
Course on genetic resources and plant genetics

1997-98 : Sabbatical, Max-Planck Institut fuer Zuechtungsforschung, Koeln,
Germany

1999 (March) : Università degli Studi, Perugia
Visiting Professor
Lectures on *Phaseolus* genetic diversity and genetics

1999-2001 : Chair, Department of Agronomy and Range Science, University of
California, Davis

E. AWARDS:

Graduated with the highest honors, Faculté des Sciences Agronomiques, Gembloux, Belgium

Fellowship of the Belgian American Educational Foundation, New Haven, Connecticut, USA: 1981-82.

Distinguished Achievement Award, Bean Improvement Cooperative, 1991

Best research paper for 1991, Centro Internacional de Agricultura Tropical, Cali, Colombia (for Singh et al. 1991. Crop Sci. 31: 23-29)

Elected Fellow, American Association for the Advancement of Science, 2001

Elected member of the council, American Genetics Association, 2001

F. MEMBERSHIPS IN SOCIETIES:

American Association for the Advancement of Science, American Society for Horticultural Science, Bean Improvement Cooperative, Crop Science Society of America, Society for Economic Botany.

G. MEETINGS, SYMPOSIA, AND INVITED PRESENTATIONS:

1. Bean Improvement Cooperative: Gainesville, Florida: January 5-7, 1981
   Presentation:P. Gepts. Comparison of early generations of *Phaseolus vulgaris* x *P. coccineus* subsp. *coccineus* and *P. vulgaris* x *P. coccineus* subsp. *polyanthus* crosses.

2. American Society of Horticultural Science: Ames, Iowa: August 8-13, 1982

3. American Society of Agronomy: Washington, D.C.: August 14-19, 1983
   Presentation: P. Gepts and F.A. Bliss. Enhanced seed methionine content associated with increased levels of phaseolin, the major seed storage protein of the common bean (*Phaseolus vulgaris*).

4. Bean Improvement Cooperative: Minneapolis, Minnesota: November 8-10, 1983
   Presentation: P. Gepts and F.A. Bliss. Enhanced seed methionine content associated with higher phaseolin levels in common bean (*Phaseolus vulgaris*)
   Poster: P. Gepts, T.C. Osborn, K. Rashka, and F.A. Bliss. Phaseolin variability in the common bean (*Phaseolus vulgaris* L.): Evidence for independent domestication centers in Mesoamerica and the Andes.

5. Stadler Symposium: Columbia, Missouri: March 19-21, 1984

Poster: P. Gepts and F.A. Bliss. Two gene pools in cultivated *Phaseolus vulgaris* L.

6. Genetics Society of America: Boston, Massachussets: August 11-15, 1985

7. Meeting on tepary bean: Mexicali, Mexico: August 30, 1985

8. Stebbins Symposium on Plant Evolutionary Biology: Davis, California: September 12-14, 1986.

9. Fifth International Bean Nurseries Conference: Centro Internacional de Agricultura Tropical, Cali, Colo
Invited presentation: Patterns of genetic diversity and domestication in *Phaseolus* beans:
Implications for genetic resources and breeding.

10. Bean Improvement Cooperative: Denver, Colorado: October 26-29, 1987.
Presentation: C. Schinkel, S. Singh, and P. Gepts. Patterns of genetic diversity in *Phaseolus* beans.

11. Clause Seed Co., Brétigny-sur-Orge, France: January 5, 1988:
Invited presentation: "La variabilité génétique chez le haricot commun: importance pour l'amélioration."

12. Instituto de Biología, Universidad Nacional Autónoma de México: March 24, 1988: Invited presentation : "La diversidad genética en los frijoles *Phaseolus*."

13. Centro de Fijación de Nitrógeno, Universidad Nacional Autónoma de México, Cuernavaca, México: March 25, 1988: Invited presentation: "Polimorfismos en *Phaseolus vulgaris*."

14. UCD-INIFAP Conference: Guadalajara, México: March 28, 1988: With C. Qualset and D. Parfitt: "Plant genetic resources and crop improvement: an overview and basis for cooperation between Mexico and California."

15. International Symposium: Population Genetics and Germplasm Resources in Crop Improvement, University of California, Davis, August 11-13, 1988: Invited presentation: "Seed storage protein diversity in plants."

16. American Institute for Biological Sciences, Symposium on New Perspectives on the Origin and Evolution of Selected New World Plants", co-sponsored by the Society for Economic Botany and the Economic Botany section of the American Botanical Society, University of California, Davis, August 17-18, 1988: Invited presentation: "Biochemical evidence bearing on the domestication of *Phaseolus* beans."

17. Centro Internacional de Agricultura Tropical, Cali, Colombia: March 16, 1989: Invited presentation: "An update on studies of genetic diversity in *Phaseolus*."

18. University of Wisconsin, Madison, Dept. of Horticulture: July 21, 1989. Invited presentation:

Genetic diversity of *Phaseolus* beans: what you see is not always what you get.

19. Horticultural Biotechnology Symposium, University of California, Davis, August 21-23, 1989: Poster (with S. Singh, A. Gutiérrez, C. Urrea, C. Schinkel, R. Koenig, R. Nodari): Marker-based analysis of genetic diversity in common bean.

20. Bean Improvement Cooperative Meeting, Toronto, November 7-9, 1989: Presentation (with S. Singh, A. GutiJrrez, C. Urrea, R. Koenig, and R. Nodari): Marker-based analysis of egenetic diversity in common bean.
    Poster (with G.C. Arndt): Inheritance of heat tolerance in common bean.

21. UCLA Symposium, Molecular Strategies for Crop Improvement, April 16-22, 1990; Poster (with Shree Singh, Rubens Nodari, Belén Garrido, and Epimaki Koinange): Towards an integrated linkage map of common bean (*Phaseolus vulgaris* L.).

22. Washington State University (IAREC), Prosser, WA, May 17, 1990: Invited presentation: The use of molecular markers in genetic studies of common bean, *Phaseolus vulgaris*

23. Colegio de Ingenieros Agrónomos de Loja y Zamora-Chinchipe, Loja, Ecuador, June 22, 1990: Invited presentation: Domesticación de cultivos y recursos fitogenéticos (Domestication of cultivated plants and plant genetic resources)

24. Estación Experimental Agrícola de Santa Catalina, INIAP, Quito, Ecuador, July 2, 1990: Invited presentation: La domesticación del frijol: implicaciones para los recursos fitogenéticos y el mejoramiento. (Domestication of common bean: implications for genetic resources and breeding).

25. Centro Internacional de Agricultura Tropical, Cali, Colombia, September 11-14, 1990: Invited presentation: Improved characterization of *Phaseolus vulgaris* genetic variability.

26. Ninth SUA/WSU Bean/Cowpea CRSP and 2nd SADCC/CIAT Regional Bean Research Workshop, Sokoine University, Morogoro, Tanzania, September 17-22, 1990: Invited presentation: Genetic resources available in *Phaseolus vulgaris*

27. American Society of Agronomy 1990 Annual Meeting, San Antonio, October 21-26, 1990: Presentation: with S.P. Singh, J.A. Gutiérrez, A. Molina, C. Urrea: Marker-based classification of genetic diversity in common bean

28. American Society of Agronomy 1990 Annual Meeting, San Antonio, October 21-26, 1990: Presentation: by R. Nodari (graduate student), S.P. Singh, P. Gepts : RFLPs and linkage mapping in common bean

29. American Society of Agronomy 1990 Annual Meeting, San Antonio, October 21-26, 1990: Presentation: by G. Arndt (graduate student), P. Gepts: Inheritance study of heat tolerance in common bean

30. American Society of Agronomy 1990 Annual Meeting, San Antonio, October 21-26, 1990:
Poster: by L. Panella (graduate student), P. Gepts: Variation in seed storage protein of
wild and cultivated cowpeas

31. University of California, Davis, Plant Biology Graduate Group
Seminar presentation, December 7, 1990: Germplasm exploration for wild beans in
Ecuador

32. Michigan State University, East Lansing, MI. Mini-Symposium on Molecular markers in
plant genetics and crop evolution, Invited presentation, December 14, 1990: Molecules,
phenotypes, and multivariate analyses in *Phaseolus* evolution

33. North Carolina State University, Symposium on Plant Breeding in the 1990s, Poster
presentation, with R. Nodari: Linkage map of common bean (*Phaseolus vulgaris* L.)

34. North Carolina State University, Symposium on Plant Breeding in the 1990s, Poster
presentation, with S.P. Singh, J.A. GutiJrrez, A. Molina, C.A. Urrea: Potential of wild
common bean populations for improvement of cultivars in the tropics.

35. 13th North American Symbiotic Nitrogen Fixation Conference 1991, Banff, Canada, August
25-30, 1991: Poster presentation with S.M. Tsai, R.O. Nodari, P.A. Arraes, E. Koinange:
RFLP analysis of bean (*Phaseolus vulgaris* L.) cultivars selected for nitrogen fixation.

36. AID/PSTC networking meeting, Banff, Canada, September 1-6, 1991. Presentation, with S.P.
Singh: Establishing a core collection for *Phaseolus vulgaris* genetic resources
conservation.

37. AID/PSTC networking meeting, Banff, Canada, September 1-6, 1991. Invited plenary
presentation: Usefulness of molecular identification of plant selections and
microorganisms.

38. UC Davis, Dept. of Pomology: Variations in common bean: potential implications for
breeding and disease resistance management

39. Ferry-Morse Seed Co., Hollister, CA: December 4, 1991: Invited presentation: The potential
of RAPD markers in breeding

40. Miami Bio/Technology Winter Symposia: January 20-24, 1992: Poster, selected for short
presentation: with R.O. Nodari and S.M. Tsai, Locating genetic factors for nodulation
intensity on the RFLP map of common bean (*Phaseolus vulgaris*)

41. UCD-Pacific Rim Food and Agricultural Biotechnology Conference: Sacramento, June 20-
24, 1992: Poster presentation, with R.L. Gilbertson, R.O. Nodari, P. Guzmán, and S.M.
Tsai, Mapping genetic factors controlling host-bacteria interactions in common bean.

42. Third International Legume Conference, Kew, U.K., July 12-17, 1992: Invited Presentation: Origin and Evolution of Cultivated *Phaseolus* Species

43. Academia Brasileira de Ciência, Rio de Janeiro, July 24, 1992: Presentation by J. Dobereiner: S.M. Tsai, R.O. Nodari, and P. Gepts: Analise quantitativa e mapeamento de genes da nodulaçao em feijoeiro (*Phaseolus vulgaris* L.) [Quantitative analysis and mapping of nodulation genes in common bean (*Phaseolus vulgaris* L.)]

44. American Phytopathological Society Annual Meeting, 1992: Poster presented by P. Guzmán, with D. Mandala, R. Nodari, W.A.B. Msuku, A.B.C. Mkandawire, P. Gepts, S. Temple, and R.L. Gilbertson: Differentiation of the angular leafspot fungus with RAPD markers: evidence for coevolution with the common bean.

45. International Board for Plant Genetic Resources-CENARGEN-CNG, Brasilia: August 23-29, 1992: Invited presentation: Genetic markers and core collections

46. Societá Italiana di Genetica Agraria, Metaponto, Matera, Italy, Oct 5-8, 1992: Poster presentation by Sonnante G, with Stockton T, Gepts P (1992) Fingerprinting del DNA in *Phaseolus vulgaris* L.

47. American Society of Agronomy - American Society for Horticultural Science Joint Symposium on Applications of RAPD Technology to Plant Breeding, Minneapolis, Nov. 1, 1992: Invited Presentation: Use of Hypervariable Markers

48. 9th International Congress on Nitrogen Fixation, Cancún, Mexico, Dec. 6-12, 1992: Poster Presentation by S.M. Tsai, with R.O. Nodari, P. Gepts: The heritability of nodule formation in common bean (*Phaseolus vulgaris* L.)

49. 9th International Congress on Nitrogen Fixation, Cancún, Mexico, Dec. 6-12, 1992: Poster Presentation by S.M. Tsai, with R.O. Nodari, P. Gepts: QTL analysis and mappping nodulation genes in common bean (*Phaseolus vulgaris* L.)

50. California Seed Association, San Diego, May 6, 1993: Invited presentation: Molecular markers for the seed industry.

51. DCB/UCD Biotechnology Symposium, Lake Tahoe, August 2-4, 1993: Invited presentation: Molecular linkage map analysis of the inheritance of genetically complex traits

52. University of Basel, Switzerland, September 22, 1993: Invited presentation: Genome map analysis of quantitative traits

53. Societá Italiana di Genetica Agraria, Orvieto, Italy, October 11-14, 1993: Poster presentation by M. Frediani, with R. Bernardi, P. Gepts, P. Cionini: Localizzazione cromosomica di gruppi di concatenazione genetica in *Phaseolus*.

54. Bean Improvement Cooperative, November 2-4, 1993: Oral presentation: Gene transmission in Middle american x Andean crosses of common bean.

55. Workshop on *in situ* conservation, CIMMYT, México, December 16, 1993: Invited presentation: Characterization of genetic diversity in common bean using molecular markers

55. University of Paris and Institut National de la Recherche Agronomique, Ferme du Moulon, Orsay, January 13, 1994: Invited presentation: La génétique de la domestication chez le haricot (*Phaseolus vulgaris* L.)

56. Plant Genome II Conference, San Diego, January 23-27, 1994. Poster: with E.M.K. Koinange and S.P. Singh: Molecular Linkage Map Analysis of the Genetics of Domestication in Common-Bean

57. Plant Genome II Conference, San Diego, January 23-27, 1994. Poster: with O.M. Paredes: Variation for segregation and linkage in crosses of common-bean

58. Plant Genome II Conference, San Diego, January 23-27, 1994. Poster: with V. Llaca: Structure and organization of the phaseolin multigene family in *Phaseolus vulgaris*

59. American Society for Horticultural Science, Corvallis, August 7-11, 1994: Invited presentation: Organization of genetic diversity in common bean (*Phaseolus vulgaris*): implications for genetic conservation and breeding

60. American Society for Horticultural Science, Corvallis, August 7-11, 1994: Presentation: What does it ake to get a cultivated bean?

61. XI Latin American Congress Genetics & XV Mexican Congress Plant Genetics, Monterrey, Mexico, 25-30 September, 1994: Plenary lecture: Análises moleculares del proceso de domesticación en plantas: el ejemplo del fríjol común (*Phaseolus vulgaris*).

62. Plant Genome III Conference, San Diego, January 1995: Poster: with R Freyre, Skroch P, Adam-Blondon AF, Shirmohamadali A, Johnson WC, Nodari RO, Koinange EMK, Sevignac M, Bannerot H, Nienhuis J Integrated linkage map of common bean (*Phaseolus vulgaris* L.)

63. Plant Genome III Conference, San Diego, January 1995: Poster: with Asgar Shirmohamadali : Analysis of pollen DNA by PCR in common bean

64. Plant Genome III Conference, San Diego, January 1995: Poster: with William C. Johnson and Paul Gepts: The association of size differences with seed storage protein class in common beans (*Phaseolus vulgaris*): Sax's hypothesis revisited

65. Plant Genome III Conference, San Diego, January 1995: Poster: with Cristina Menéndez, Anthony E. Hall: A genetic linkage map of cowpea (*Vigna Unguiculata* (L.) Walp) based on RAPD markers.

66. Plant Genome III Conference, San Diego, January 1995: Poster: with VRctor Llaca: Relationship between physical and genetic distances in the phaseolin region of *Phaseolus vulgaris*.

67. Colorado State University, Fort Collins, October 12, 1995: Invited presentation

68. American Society of Agronomy, St. Louis, October 31, 1995: Invited presentation: with R. Freyre: The Origin and Evolution under Domestication of *Phaseolus*

69. CENA, Universidade de São Paulo, Piracicaba, December 15 and 21, 1995: Invited presentations: Molecular analyses of the origin of *Phaseolus vulgaris*

70. Universidade Federal de Viçosa, Viçosa, Brazil: December 19: Invited presentation

71. Plant Genome IV Conference, San Diego, January 14-18, 1996: Invited presentation: with Rosanna Freyre, William C. Johnson, Asghar Shirmohamadali, Victor Llaca, Paul Skroch, James Nienhuis, Anne-Francoise Adam-Blondon, Michel Dron: Genome Distribution of Disease Resistance Genes in Common Bean

72. Plant Genome IV Conference, San Diego, January 14-18, 1996: With W. Johnson: Characterization of reproductive isolation barriers in inter- gene pool crosses of common bean (*Phaseolus vulgaris* L.)

73. Michigan State University, East Lansing, MI, December 13, 1996: Invited presentation: Origin and evolution of *Phaseolus vulgaris*: do we know beans?

74. Plant and Animal Genome V, San Diego, January12-16, 1997: with W.C. Johnson: Poster: Localization of genomic regions conditioning performance and seed size in inter-gene pool crosses of common bean (*Phaseolus vulgaris* L.)

75. International Symposium, The origins of agriculture and the domestication of crop plants in the Near East: ICARDA, Aleppo, Syria, May 10-14, 1997: Presentation: Molecular analysis of the domestication process in common bean

76. American Society for Horticultural Science, Annual Meeting, Salt Lake City: July 24, 1997. Invited presentation: with W.C. Johnson: Influence of diverse evolution in Phaseolus and prospects for bean gene pool enrichment

77. International Plant Genetics Resources Institute, Rome, Italy: September 22, 1997. Invited presentation: Lessons from *Phaseolus vulgaris* germplasm studies

90. University of Georgia, Dept. of Genetics, Jan. 31, 2000: Invited presentation: Investigations of Gene Flow in Phaseolus vulgaris, a Predominant Selfer

91. University of Georgia, Dept. of Botany, Jan. 31, 2000: Invited presentation: Phylogeographic Analyses of Plant Domestication

92. CICY, Mérida, Yucatán, Mexico: June 30, 2000: Invited presentation: Análises moleculares del proceso de domesticación en frijol.

93. Monterey County, CA; Biotechnology Conference: October 20, 2000: Invited presentation: Potential Environmental Concerns Associated with Transgenics

94. American Society of Agronomy, Minneapolis: November 9, 2000: Invited presentation: Gene Flow between Wild and Cultivated *Phaseolus vulgaris*

95. Université de Montpellier II: December 5, 2000: Invited presentation: Une analyse phylogéographique et génomique de la diversité génétique chez le haricot (*Phaseolus vulgaris*)

96. Plant and Animal Genome Meeting IX: January 13-17, 2001: Poster presentation (with James Kami, Valérie Geffroy, Valérie Poncet): Development of a set of phylogenetically informative bac libraries in the genus *Phaseolus*

. Plant and Animal Genome Meeting IX: January 13-17, 2001: Poster presentation (with Rian Lee, Mark Bassett, Phillip McClean): STS and RAPD mapping of genes controlling seedcoat patterning and color in common bean (*Phaseolus vulgaris* L.)

98. Plant and Animal Genome Meeting IX: January 13-17, 2001: Poster presentation (with Valérie Geffroy, Elodie Ferrier Cana, Mireille Sevignac, Francine Creusot, Catherine Macadre, Catherine Grandclement, Thierry Langin): Molecular analysis of paralogs of a resistance gene cluster in two representative genotypes of the major gene pools of *Phaseolus vulgaris*

99. American Association for the Advancement of Science: February 17: Symposium on crop domestication: Invited presentation: Questions Raised by the Use of Molecular Information in Domestication Studies

100. Cornell University: Department of Plant Breeding: February 25-27, 2001: Invited Presentation: A map-based analysis of molecular diversity and gene flow in common bean

101. First Meeting of the Phaseolus Genome Initiative, Cuernavaca, March 3-4, 2001: *Phaseolus-Vigna* as Genetic Organisms

. Rhizosphere Biology Workshop, Davis,CA: June 1-3, 2001: Poster presentation: Effect of

77. Società Italiana di Genetica Agraria, Annual meeting, Abadia di Fiastra, Macerata, September 24-26, 1997. Invited presentation: A molecular analysis of the origin of common bean and evolutionary factors affecting its diversity

78. 2nd International Ethnobotany Congress, Mérida, Mexico: October 13-17, 1997. Invited presentation: El origen del frijol común y su diversificación bajo domesticación

79. Max-Planck-Institute for Plant Breeding Research, November 5, 1997. Institute seminar: The genetics of domestication in common bean, *Phaseolus vulgaris* L.

80. Iowa State University, Ames, Iowa: Plant Science Lectures, Jan. 8-9, 1998: Invited presentation: Wild relatives of crops: an underestimated and underutilized source of genetic diversity for crop improvement

81. Plant and Animal Genome VI, San Diego, Jan. 18-22, 1998: Workshop presentation: by W.C. Johnson: Influence of epistasis and diverse evolution on wide crosses of common bean

82. Plant and Animal Genome VI, San Diego, Jan. 18-22, 1998: Poster presentation: by R. Papa, with J. Acosta, A. Delgado: Gene flow between wild and cultivated *Phaseolus vulgaris* L. in Mexico: potential for gene escape from transgenic plants into wild population?

83. Eucarpia Symposium on Breeding of Oil and Protein Crops, Pontevedra, Spain, April 1-4, 1998: Invited presentation: Using phaseolin seed protein to understand germplasm diversity in common bean (*Phaseolus vulgaris*).

84. Stadler Symposium, Columbia, Missouri, June 8-10, 1998: Invited presentation: A phylogenetic and genomic analysis of crop germplasm: a necessary condition for its rational conservation and use.

85. Novartis Foundation, London, June 26, 1998: Discussion on DNA and agricultural origins

86. International Organization of Plant Biosystematists, VIIth International Symp., Amsterdam. August 10-15, 1998: Human influences during and after domestication of *Phaseolus*

87. National Institute of Agrobiological Resources (NIAB), Ministry of Agriculture, Forestry, and Fisheries (MAFF), Tsukuba, October 13-15, 1999: Invited presentation: with R. Papa, S. Coulibaly, and R. Pasquet, Wild Legume Diversity and Domestication - Insights from Molecular Methods

88. American Society of Agronomy, Annual Meeting, Salt Lake City: Oct. 31-Dec. 4, 1999: with R. Papa, A. Gonzalez-Mejia, A. Wong, J. Acosta, A. Delgado Salinas, Gene flow in *Phaseolus vulgaris*.

89. Bean Improvement Cooperative, Biennial meeting, Calgary: Nov. 13-15, 1999: Outcrossing in Mexican wild and domesticated populations of common bean.

host and bacterial genotypes on nodulation in the *Phaseolus-Rhizobium* and disease development in the *Phaseolus-Xanthomonas* Interactions

102. *Medicago truncatula* workshop, Madison, WI: July 7-9, 2001: Invited presentation: *Phaseolus* genomics

103. Forest tree biotechnology meeting, Stevenson, WA: July 22-27, 2001: Invited presentation: The inheritance of the domestication syndrome in field crops: are there lessons for tree crops?

104. CGIAR-U.S Universities Initiative in Legume Genomics: August 21-22, 2001: Invited presentation: Legume Evolution

105. American Society of Agronomy, Charlotte, NC: October 21-25, 2001: Invited presentation: Exploration of *Phaseolus* germplasm: a dynamic interplay between field and lab activities

106. Bean Improvement Cooperative, Fargo, ND: October 29- November 2, 2001: Invited presentation: Genomics research in *Phaseolus*: what's in it for you?

107. New York State Museum, Albany, NY: 2002 Northeast Natural History Conference, Symposium on The History and Impacts of Maize-Bean-Squash Agriculture: Invited presentation: Origin and Dispersal of Common Bean (*Phaseolus vulgaris* L.): April 24-27, 2002

108. Second International Phaseomics (Phaseolus Genomics) Consortium Meeting, Geneva, Switzerland; May 16-19, 2002: Invited presentation: Opportunities for Evolutionary Genomic Studies in *Phaseolus*

109. First International Conference on Legume Genomics and Genetics, Minneapolis, MN, June 2-6, 2002 : Invited presentation: The Origin and Evolution of Phenotypic Diversity in Common Bean

110. University of Illinois, Champaign-Urbana, IL: Symposium on Long-term Selection: A celebration of 100 generations of selection for oil and protein in maize: June 17–19, 2002: Invited presentation: Domestication as a Long-Term Selection Experiment

111. Society for Economic Botany Annual Meeting, NY Botanical Garden, NY: June 23-26, 2002: Invited presentation: Phylogeographic studies in *Phaseolus*

H. PUBLICATIONS: refereed unless indicated otherwise

1. 1977 Gepts, P., Angarita, A., Kummert, J. Labeling of the replicative forms of three Tymoviruses. Parasitica 33: 95-102.

2.   1977   Kummert, J., Gepts, P., Angarita-Zerda, A. Comparison between the RNAs of three Tymoviruses as studied by competitive molecular hybridization. Parasitica 33: 147-54.

3.   1978   Meeus, P., Fraselle, J., Gepts, P., Froidmont, F. Essais de lutte chimique contre la rynchosporiose (Rynchosporium secalis (Oud.) Davis) de l'escourgeon. Parasitica 34 35-48.

4.   1980   Gepts, P. Variability from interspecific hybridisations. In: Bean Program 1979 Annual Report, CIAT, Cali, Colombia: pp. 28-34. (not refereed)

5.   1980   Hidalgo, R., Song, L., Gepts, P. Diversidad genética de la especies cultivadas de Phaseolus. Autotutorial unit, CIAT, Cali, Colombia: 52p. (not refereed)

6.   1981   Gepts, P. Variability from interspecific hybridisations. In: Bean Program 1980 Annual Report, CIAT, Cali, Colombia: pp. 30-32. (not refereed)

7.   1981   Gepts, P. Hibridaciones interespecíficas para el mejoramiento de Phaseolus vulgaris L. CIAT, Cali, Colombia, Serie SE-10-81: 17p. (not refereed)

8.   1982   Gepts, P. Variability from interspecific hybridisations. In: Bean Program 1981 Annual Report, CIAT, Cali, Colombia: pp. 66-70. (not refereed)

9.   1982   Fernandez, F., Gepts, P., Lopez, M. Etapas de desarrollo de la planta de frijol comdn. Autotutorial unit, CIAT, Cali, Colombia: 26p. (not refereed)

10. 1984   Gepts, P., Bliss, F.A. Enhanced available methionine concentration associated with higher phaseolin levels in common bean seeds. Theor. Appl. Genet. 69: 47-53.

11. 1985   Gepts, P., Bliss, F.A. F1 hybrid weakness in the common bean: differential geographic origin suggests two gene pools in cultivated common bean germplasm. J. Heredity 76: 447-450.

12. 1986   Gepts, P., Bliss, F.A. Phaseolin variability among wild and cultivated common beans from Colombia. Econ. Bot. 40: 469-478.

13. 1986   Gepts, P., Osborn, T.C., Rashka, K., Bliss, F.A. Electrophoretic analysis of phaseolin protein variability in wild forms and landraces of the common bean, Phaseolus vulgaris : Evidence for multiple centers of domestication. Econ. Bot. 40: 451-468.

14. 1986   Osborn, T.C., Blake, T.K., Gepts, P., Bliss, F.A. Bean arcelin protein. II. Genetic variation, inheritance, and linkage relationships in common bean. Theor. Appl. Genet. 71: 847-851.

15.   1987   Gepts, P. Wild ancestors of crop plants - A neglected resource. J. Washington Acad. Sci. 4:

16.   1987   Gepts, P. Characterizing plant phenology: growth and development scales. In: K. Wisiol and J.D. Hesketh (eds.), Plant growth modeling for resource management, 2 vols., CRC Press, Boca Raton, Florida, USA, Vol. 2: pp. 4-24.

17.   1988   Gepts, P., Kmiecik, K., Pereira, P., and Bliss, F.A. Dissemination pathways of common bean (Phaseolus vulgaris, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. Econ. Bot. 42: 73-85.

18.   1988   Gepts, P., and Bliss, F.A. Dissemination of common bean (Phaseolus vulgaris, Fabaceae) deduced from phaseolin electrophoretic variability. II. Europe and Africa. Econ. Bot. 42: 86-104.

19.   1988   Gepts, P. Phaseolin as an evolutionary marker. In: P. Gepts (ed.), Genetic Resources of Phaseolus beans, Kluwer, the Netherlands: pp. 215-241.

20.   1988   Gepts, P. A Middle American and an Andean common bean gene pool. In: P. Gepts (ed.), Genetic Resources of Phaseolus beans, Kluwer, the Netherlands: pp. 375-390.

21.   1988   Delgado Salinas, A., Bonet, A., Gepts, P. The wild relative of Phaseolus vulgaris in Middle America. In: P. Gepts (ed.), Genetic Resources of Phaseolus beans, Kluwer, the Netherlands: pp. 163-184.

22.   1988   Schinkel, C., and Gepts, P. Phaseolin diversity in the tepary bean, Phaseolus acutifolius A. Gray. Plant Breeding 101: 292-301.

23.   1989   Gepts, P. and M.T. Clegg. Genetic diversity in pearl millet (Pennisetum glaucum (L.) R. Br.) at the DNA sequence level. J. Hered. 80: 203-208.

24.   1989   Schinkel, C., Gepts, P. Allozyme variability in the tepary bean, Phaseolus acutifolius A. Gray. Plant Breeding 102: 182-195.

25.   1989   Gepts, P. Bean. McGraw-Hill 1990 Yearbook of Science and Technology: pp. 38-39 (not refereed)

26.   1989   Gepts, P. Genetic diversity of seed storage proteins in plants, pp. 64-82. In: A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir (eds.), Population Genetics and Germplasm Resources in Crop Improvement. Sinauer, Sunderland, MA.

27.   1989   Koenig, R., Gepts, P. Segregation and linkage of genes for seed proteins,
              isozymes, and morphological traits in common bean (Phaseolus vulgaris).
              J. Heredity 80: 455-459.

28.   1989   Koenig, R., Gepts, P. Allozyme diversity in wild Phaseolus vulgaris: further
              evidence for two major centers of genetic diversity. Theor. Appl. Genet.
              78: 809-817

29.   1989   Gepts, P. Diversidad genética y domesticación en el género Phaseolus, y sus
              implicaciones, pp. 379-396. In: Progreso en la investigación y producción
              del frijol común (Phaseolus vulgaris L.). Centro Internacional de
              Agricultura Tropical, Cali, Colombia. (Not refereed)

30.   1989   Singh, S.P., Debouck, D.G., Gepts, P. Races of common bean, Phaseolus vulgaris
              L. In S. Beebe (ed.), Current topics in breeding of coommon bean.
              Working Document No. 47, Bean Program, Centro Internacional de
              Agricultura Tropical, Cali, Colombia: pp. 75-89. (Not refereed)

31.   1990   Koenig, R. Singh, S.P., Gepts, P. Novel phaseolin types in wild and cultivated
              common bean (Phaseolus vulgaris, Fabaceae). Econ. Bot. 44: 50-60.

32.   1990   Gepts, P. Biochemical evidence bearing on the domestication of Phaseolus
              (Fabaceae) beans. Econ. Bot. 44 (3S): 28-38.

33.   1991   Gepts, P., Debouck, D.G. Origin, domestication, and evolution of the common
              bean, Phaseolus vulgaris, pp. 7-53. In: O. Voysest and A. Van
              Schoonhoven, eds., Common beans: research for crop improvement,
              CIAT, Cali, Colombia.

34.   1991   Singh, S.P., Nodari, R. Gepts, P. Genetic diversity in cultivated Phaseolus
              vulgaris. I. Allozymes. Crop Sci. 31: 19-23.

35.   1991   Singh, S.P., Gutiérrez, J.A., Molina, A., Urrea, C., Gepts, P. Genetic diversity in
              cultivated Phaseolus vulgaris. II. Marker-based analysis of morpho-
              agronomic diversity. Crop Sci. 31: 23-29.

36.   1991   Garrido, B., Nodari, R., Debouck, D.G., Gepts, P. Uni-2, a dominant mutation
              affecting leaf development in Phaseolus vulgaris. J. Hered. 82: 181-183.

37.   1991   Singh, S.P., Gepts, P, Debouck, D.G. Races of common bean, (Phaseolus
              vulgaris, Fabaceae). Econ. Bot. 45: 379-396.

38   1991   Gepts P. Book review on "Tissue culture in leguminous and oilseed crops", edited
              by Y.P.S. Bajaj. Springer. Trends Biotech.

39.   1992   Koinange, E.M.K., Gepts, P. Hybrid weakness in wild *Phaseolus vulgaris* L. J. Hered.83:135-139

40.   1992   Stockton T, Sonnante G, Gepts P. Detection of minisatellite sequences in *Phaseolus vulgaris*. Plant Molec. Biol Rept. 10:47-59

41.   1992   Nodari, R.O., Koinange, E.M.K., Kelly, J.D., Gepts, P. Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor. Appl. Genet. 84: 186-192

42.   1992   Gepts P, Llaca V, Nodari RO, Panella L. Analysis of seed proteins, isozymes, and RFLPs for genetic and evolutionary studies in *Phaseolus*. *In*: Linskens HF, Jackson JF (eds), Seed analysis, Modern Methods of Plant Analysis, Vol. 14. Springer, Berlin: pp. 63-93.

43.   1992   Panella L, Gepts P. Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses. Genet. Res. Crop Evol. 39:71-88.

44.   1993   Nodari RO, Tsai SM, Gilbertson RL, Gepts P. Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. Theor. Appl. Genet. 85: 513-520

45.   1993   Gepts P. Linkage map of common bean (Phaseolus vulgaris L.). *In*: O'Brien SJ (ed), Genetic Maps, 6th edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 6.101-6.109

46.   1993   Nodari RO, Tsai SM, Guzm<n P, Gilbertson RL, Gepts P. Towards an Integrated Linkage Map of Common Bean. 3. Mapping genetic factors controlling host-bacterium interactions. Genetics 134: 341-350.

47.   1993   Gepts P, Nodari R, Tsai SM, Koinagne E.M.K. , Llaca V, Gilbertson R, Guzman P. Linkage Mapping in Common Bean. Annu. Rept. Bean Improv. Coop. 93: xxiv-xxxv (not refereed)

48.   1993   Gepts P. The use of molecular and biochemical markers in crop evolution studies. Evol. Biol. 27, 51-94

48.   1993   Panella L, Kami J, Gepts P. Vignin diversity in wild and cultivated taxa of *Vigna unguiculata* (L.) Walp. (Fabaceae). Econ. Bot. 47: 371-386

49.   1993   Debouck DG, Toro O, Paredes O, Johnson WC, Gepts P. Genetic diversity and ecological distribution of *Phaseolus vulgaris* on northwestern South America. Econ. Bot. 47: 408-423

49. 1994 Becerra Velásquez VL, Gepts P. RFLP diversity in common bean. Genome 37: 256-263

50. 1994 Llaca V, Delgado-Salinas A, Gepts P. cpDNA as an evolutionary marker in the *Phaseolus vulgaris* complex. Theor. Appl. Genet. 88:646-652

60. 1994 Shónnard GC, Gepts P. Genetics of heat tolerance during reproductive development in common bean. Crop Sci. 34: 1168-1175

61. 1994 Sonnante G, Stockton T, Nodari RO, Becerra Velásquez VL, Gepts P. Evolution of genetic diversity during the domestication of common-bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 89: 629-635

62. 1994 Kami J, Gepts P. Phaseolin nucleotide sequence diversity in *Phaseolus*. I. Intraspecific diversity in *Phaseolus vulgaris*. Genome 37:751-757

63. 1994 Stockton T, Gepts P. Identification of DNA probes that reveal polymorphisms among closely related *Phaseolus vulgaris* lines. Euphytica 76:177-183

64. 1995 Guzmán P, Gilbertson RL, Nodari RL, Johnson WC, Temple S, Mandala D, Mkandawire ABC, Gepts P. Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). Phytopathology 85:600-607

65. 1995 Gutiérrez Salgado A, Gepts P., and Debouck D.G. Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. Genet. Res. Crop Evol. 42: 15-28

66. 1995 Paredes OM, Gepts P. Extensive introgression of Middle American germplasm into Chilean common bean cultivars. Genet. Res. Crop Evol. 42: 29-41

67. 1995 Kami J, Becerra Velásquez V, Debouck DG, Gepts P. Identification of the presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proc. Nat. Acad. Sci. 92: 1101-1104

68. 1995 Paredes OM, Gepts P. Segregation and recombination in inter-gene pool crosses of *Phaseolus vulgaris* L. J. Hered. 86: 98-106

69. 1995 Gepts P. Genetic markers and core collections. *In*: Brown AHD, van Hintum T, Hodgkin T, Morales EAV (eds.), Core collections: improving the management and use of plant germplasm collections. Wiley, NY: pp. 127-146.

70. 1995 Singh SP, Molina A, Gepts P. Potential of wild common bean for seed yield improvement of cultivars in the tropics. Can. J. Plant Sci. 75: 807-813

71. 1996 Freyre R, Ríos R, Guzmán L, Debouck DG, <u>Gepts P</u>. Ecogeographic distribution
of *Phaseolus* spp. (Fabaceae) in Bolivia. Econ. Bot. 50: 195-215

72. 1996 Koinange EMK, Singh SP, <u>Gepts P</u>. Genetic Control of the domestication
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73. 1996 Johnson WC, Menéndez C, Nodari R, Koinange EMK, Magnusson S, Singh SP,
<u>Gepts P</u>. 1996. Association of a seed weight factor with the phaseolin seed
storage protein locus across genotypes, environments, and genomes in
Phaseolus-Vigna spp.: Sax (1923) revisited. J. Quant. Trait Loci, Volume
2, Article 5. http://probe.nalusda.gov:8000/otherdocs/jqtl.

74. 1996 <u>Gepts P</u>, Freyre RO. The use of biochemical and molecular markers in
elucidating the origin and evolution of *Phaseolus* beans. In: D.
Grattapaglia and M. Ferreira (eds.), Molecular markers in biodiversity,
genetics and breeding of plants - current techniques and their applications,
EMBRAPA-CENARGEN, Brasilia

75. 1996 Llaca V, <u>Gepts P</u>. Pulsed field gel electrophoresis analysis of the phaseolin locus
region in *Phaseolus vulgaris*. Genome 39, 722-729

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Lock JM (eds.) Advances in Legume Systematics, Vol 8, Legumes of
economic importance. The Royal Botanic Gardens, Kew, UK: pp. 65-74

77. 1997 Johnson WC, Guzmán P, Mandala D, Mkandawire ABC, Temple S, Gilbertson
RL, and <u>Gepts P</u>. Molecular tagging of the *bc-3* gene for introgression into
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K. CURRENT AND PAST GRADUATE STUDENTS, POSTDOCS and VISITING SCIENTISTS:

MS degree students:
1. Rosalie Koenig (USA) -- MS in International Agricultural Development -- Graduated August 1988 -- Thesis: Phaseolin and isozyme diversity in wild *Phaseolus vulgaris* L. Current occupation: Graduate student, PhD in Plant Pathology, University of Florida

2. Belén Garrido (Spain) -- M.S. in Agronomy -- Graduated June 1990 -- Thesis: Genetics of a unifoliolate mutant in *Phaseolus vulgaris*. Current occupation: Bilingual teacher, Oakland (CA) School District

3. Viviana Becerra (Chile) -- M.S. in Agronomy -- Graduation: June 1992 -- Thesis: RFLPs in *Phaseolus vulgaris*. Current occupation: Faculty, Universidad Católica de Chile, Chillán, Chile.

4. Fana Sylla (Senegal) — MS Agronomy -- Graduation: December 1998 -- Thesis: RAPD diversity in cowpea —. Current occupation: Researcher, Institut Senegalais de Recherche Agronomique, Dakar, Senegal

5. Asghar Shirmohamadali (Iran) — MS in Genetics – Graduation: December 1998.

6. Timothy Wills (USA; started Fall 2001) – MS in Horticulture and Agronomy

7. Matthew Hufford (USA; started Fall 2002) – MS in International Agricultural Development

PhD degree students:

1. Gisela Arndt (USA) -- Ph.D. in Genetics -- Thesis: Genetics of heat tolerance in common bean -- Graduation Summer 1991- Current occupation: homemaker

2. Rubens Nodari (Brazil) -- Ph.D. in Genetics ---- Thesis: Mapping of the common bean genome – Graduation December 1991 -- Current occupation: Faculty, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil

3. Lee Panella (USA) -- Ph.D. in Genetics -- Thesis: Genetic diversity of cowpea (*Vigna unguiculata*) -- Graduation Summer 1992 — Current occupation: Research geneticist, USDA-ARS, Colorado State University, Fort Collins, CO

4. Jim Kami (USA) -- Ph.D. in Genetics -- Thesis: Molecular evolution of phaseolin -- Graduation Summer 1992 — Current occupation: Postgraduate researcher, University of California, Davis, CA

5. Epimaki Koinange (Tanzania) -- Ph.D. in Genetics -- Thesis: Genetics of domestication in common bean -- Graduation Summer 1992 — Former occupation: Leader, Coffee research, Ministry of Agriculture, Lyamungu, Tanzania. Deceased.

6. Mario Paredes (Chile) -- Ph.D. in Genetics -- Thesis: Transmission genetics in inter-gene pool crosses of *Phaseolus vulgaris* — Graduation Spring 1993. Current occupation: Leader plant research, INIA, Chile

7. Victor Llaca (Mexico) -- Ph.D. in Genetics -- Thesis: Genetic diversity and molecular evolution of *Phaseolus coccineus* — Graduation spring 1994 -- Current occupation: National Cancer Institute, Bethesda, MD

8. William C. Johnson (USA)-- PhD in Genetics — Thesis: Molecular markers as tools in bean

breeding — Graduation: Summer 1997 – Current occupation: Squash
breeder, Seminis, Woodland, CA

10. Maria Rojas (Costa Rica) — PhD in Plant Biology — Thesis: Host-virus relationships in
common bean — Graduation: Spring 1998 – Current occupation: Research
associate, Department of Plant Pathology, University of California, Davis.

11. Sylvaine Coulibaly (Ivory Coast) — PhD in Genetics — Thesis: Analysis of seed protein
multigene families and AFLP diversity in cowpea – Graduation Spring
1999 – Current occupation: postdoc, Department of Vegetable Crops,
University of California, Davis

12. Margarita Mauro (Mexico; started Winter 1997) — PhD in Genetics — Thesis: Introgression
of yield genes from wild beans

13. Julianno Sambatti (Brazil; started Fall 1998) – PhD in Ecology – Thesis: Ecological genetics
of wild *Helianthus* species

14. Yonas Feleke (Ethiopia) – Visiting student of the University of Nairobi (sandwich program) -
Thesis: Genetic diversity and phylogeny of *Vigna unguiculata* and related
species (in collaboration with R. Pasquet, IRD, France)

15. Luca Pallottini (Italy) - Visiting student of the University of Padova: Genetic diversity of
beans and bean genomics

16. Myounghai Kwak (South Korea; started Fall 2002) – PhD in Genetics – Thesis: Linkage
disequilibrium

Visiting scientists and postdoctoral fellows:

1. Giambatista Polignano, Istituto di Germoplasma, Bari, Italy: July 20-September 20, 1989

2. James Kelly, Dept. of Crop and Soil Science, Michigan State University, East Lansing, MI:
September 1, 1989-August 31, 1990.

3. Tamalynn Stockton, Postdoctoral fellow from December 1, 1989 to June 15, 1992. Current
occupation: postdoctoral fellow, USDA Plant Gene Expression Center,
Albany, CA

4. Siu-Mui Tsai, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo,
Piracicaba, Brazil: September 1, 1990- February 28, 1992

5. Gabriella Sonnante, Istituto di Germoplasma, Bari, Italy: January 1, 1991- December 31, 1991.
Currrent position: researcher, Istituto di Germoplasma, Bari

6. Maria Cigales, Universidad de Colima, Mexico: January 1, 1992 - December 31, 1992.
Current postion: Faculty, Universidad de Colima

7. Rosanna Freyre, postdoctoral geneticist, September 1, 1993 - July 31, 1996. Current position:
Adjunct professor, Univ. of New Hampshire

8. Cristina Menéndez, postdoctoral geneticist, 1994-1995. Current position: University of
Pamplona, Spain

9. Roberto Papa, visiting scientist, University of Ancona, Italy, January 1996-August 1997.
Current position: Faculty, University of Ancona

10. Rémy Pasquet, IRD (previously ORSTOM), France: April 1996-April 1997. Current
location: ICIPE, Nairobi, Kenya.

11. Alonso Gonzalez, postdoc, January 1997- December 1998. Current position: CSIRO,
Darwin, Australia

12. Patricia Colunga GarciaMarin, visiting scientist, CICY, Mérida, Yucatan, Mexico, December
1998 -February 2000

13. Daniel Zizimbo, visiting scientist, CICY, Mérida, Yucatan, Mexico, December 1998 -
February 2000

14. Juan José Ferreira, Dpto. Hortofruticultura- CIATA, Villaviciosa, Asturias, Spain; October -
December 1999

15. Valérie Geffroy, visiting postdoctoral geneticist: April 2000 - July 2001. Current position:
researcher, Institut National de la Recherche Agronomique, Orsay, France

16. Valérie Poncet, visiting postdoctoral geneticist: September 2000 - August 2001. Current
position: Institut de Recherches pour le Développement, France

17. Ana Maria Torres, visiting scientist, December 2000 - February 2001. Permanent position:
Centro de Investigacion y Formacion Agraria (CIFA "Alameda del
Obispo"), Dpto. Mejora y Agronomia (Leguminosas), University of
Córdoba, Spain

18. Cristina Mapes, visiting scientist, Jardín Botánico, Universidad Nacional Autónoma de
México: January 2001 - January 2002.

Exhibit 2

Table 1. List of materials analyzed in this study

Consecutive number	CIAT No.	Name[a]	Alternate designations[a]	Country[b]	State[c]	Race[d]	Source[e]
1		Enola 2000-1		USA			ATCC
2		Canario 707		USA			Steve Temple, UC Davis
3	G02400	Mantequilla	Gentry 21953; PI312090; Mantequilla	MEX	SON	D	CIAT
4	G03273	Morado de Agua	AGS-74-B; Morado de Agua	MEX	AGS	D	CIAT
5	G03290	Flor de Mayo	AGS-88; Flor de Mayo	MEX	AGS	J	CIAT
6	G03504	Ojo de Cabra	CHIH-31; X-15267; Ojo de cabra	MEX	CHI	D	CIAT
7	G03715	Porrillo-1		ELS		M	CIAT
8	G04390	Pinto	TLAX-51; Pinto	MEX	TLX	D	CIAT
9	G04456	Jamapa		MEX	VER	M	CIAT
10	G04471	Cristal Blanco		CLE		C	CIAT
11	G04474	Coscorrón		CLE		C	CIAT
12	G04666	Magdalena 3		COL	MAG	M	CIAT
13	G04922	Rojo de Seda		HDR		M	CIAT
14	G05024	Jalo	BZL-0237; Jalo; collected 1935	BRA		N	CIAT
15	G05036	Mulatinho		BRA		M	CIAT
16	G05254	Bagajo		BRA		N	CIAT
17	G05910	Burros Grandes		CLE		C	CIAT
18	G06861	Bayo		HDR		M	CIAT
19	G07385	Uribe Redondo		COL		N	CIAT
20	G08159	Radical		COL		N	CIAT
21	G11013	Bayo		MEX	DUR	D	CIAT
22	G11295	Frijola	GTO-55-2; MEX-187; Frijola	MEX	GTO	J	CIAT

Table 1. List of materials analyzed in this study

Consecutive number	CIAT No.	Name[a]	Alternate designations[a]	Country[b]	State[c]	Race[d]	Source[e]
23	G15511	Frutilla	CLE-027; Frutilla	CLE		C	CIAT
24	G11733	Caballero		PER		P	CIAT
25	G11891	Culiacán	CULIACAN-11-57R-M-37-M-M	MEX	SIN	D	CIAT
26	G12717	Bolón Rojo		COL	NAR	P	CIAT
27	G19068	Apetito	JAL-4; PI313367; Apetito	MEX	JAL	J	CIAT
28	G13094	Mayocoba	collected 1959	MEX		D	CIAT
29	G20553	Conejo	NVRS-431; Conejo	MEX		J	CIAT
30	G19646	Quqa Pava		PER	CAJ	P	CIAT
31	G21720	Cargabello		COL		N	CIAT
32	G22041	Garbancillo Zarco		MEX		J	CIAT
33	G22215	II8FR MO-5-3-M-2-1-M		MEX			CIAT
34	G22227	MO-85-86 2598	SIN 9; MO-85-86 2598	MEX	SIN		CIAT
35	G22230	MO-85-86 2780	SIN 12; MO-85-86 2780	MEX	SIN		CIAT
36	G24554	Tórtolas Corriente		CLE			CIAT
37	G50517	G50517	OT-646; Cargamanto	COL	ANT	C	CIAT
38	G50517	Woodland Yellow		USA	NEB		Purchased by J. Kami in Woodland, CA
39		BAT93				M	CIAT
40		Jalo EEP558				N	CIAT
41		Sulphur BN142	=A	USA			J. Nienhuis and K. Kmiecik, University of Wisconsin
42		Mayocoba 1998		USA			NF&B
43		Mayocoba 2001		USA			NF&B
44		Myasi 2001		USA			NF&B
45		Frijol Canario		PER			Purchased by P. Gepts in Seattle
46		Azufrado Peruano 87		MEX			J. Acosta (INIFAP) via J. Kelly (Michigan State University)

Table 1. List of materials analyzed in this study

Consecutive number	CIAT No.	Name[a]	Alternate designations[a]	Country[b]	State[c]	Race[d]	Source[e]
47		Azufrado Regional 87		MEX			J. Acosta (INIFAP) via J. Kelly (Michigan State University)
48		Azufrado Regional 87		MEX			MPP
49		Azufrado Peruano 87		MEX			MPP
50		Azufrado Pimono78		MEX			MPP
51		Enola 2001		USA			NF&B
52		Enola 2000-2		USA			ATCC
53		Enola-NFB	=B	USA			NF&B
54		Mayocoba-NFB	=C	USA			NF&B
55		ADM	=D	USA			NF&B
56		Enola 2002	=E	USA			ATCC

[a] Entries in yellow have yellow seeds of a color similar to cv. Enola

[b] ISO country codes: BRA: Brazil; CLE: Chile; COL: Colombia; ELS: El Salvador; HDR: Honduras; MEX: Mexico; PER: Peru; USA: United States of America

[c] State, province, or department code: AGS: Aguascalientes; ANT: Antioquia; CAJ: Cajamarca; CHI: Chihuahua; DUR: Durango; GTO: Guanajuato; JAL: Jalisco; NAR: Nayarit; NEB: Nebraska; SON: Sonora; TLX: Tlaxcala; VER: Veracruz

[d] Races according to Singh et al. (1991): C: Chile; D: Durango; J: Jalisco; M: Mesoamerica; N: Nueva Granada; P: Peru

[e] Sources: INIFAP: Instituto Nacional de Investigaciones Forestales y Agropecuarias, Mexico; MPP: Manatt, Phelps, and Phillips, LLP; NF & B: Northern Feed and Bean; UC Davis: University of California, Davis

Exhibit 3

Gepts – Exhibit 3

Figure 1. Separation by electrophoresis of AFLP fragments. Each column represents a different individual.
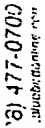
Exhibit 4

Gepts – Exhibit 4

AP78 (MEX)
AR87 (MEX)   AP87 (JA-MEX)
AR87 (MEX)   AP87 (MPP-MEX)
             Myasi 2001 (USA), ADM (USA)
I18FR (MEX) Enola-   Woodland (USA)
             (ATCC-USA) Enola-2001 (NFB-USA)
             Apetito (MEX) Culiacán (MEX)
Enola (NFB-USA)   Mayocoba-1998 (NFB-USA)
Frijol (MEX)   Mayocoba-2001 (NFB-USA)
             Mayocoba (NFB-USA)
SIN12 (MEX)

Mantequilla (MEX)
Cristal Blanco (CHL)   Bayo (MEX)
Porrillo-1 (ELS)
Jamapa (MEX)   Morado (MEX)
BAT93
Magdalena
3 (COL)   Bayo (HDR)   Sulphur (USA)   SIN 9 (MEX)   Frijol Canario (PER)
Jalo (BRA)   Mulatinho (BRA)   Ojo de cabra (MEX) Flor de Mayo (MEX)   Frutilla (CHL)
             Quda Pava (PER)   Garbancillo Zarco (MEX)   Bagajo (BRA)
Rojo de Seda   Pinto (MEX)   Canario 707 (USA)   Jalo EEP558 (BRA)
(HDR)   Mayocoba (MEX)   Uribe Redondo (COL)   Burros Grandes (CHL)
                    Cargabello (COL)   Tórtolas Corriente (CHL)
             Radical (COL)   G50517 (COL)
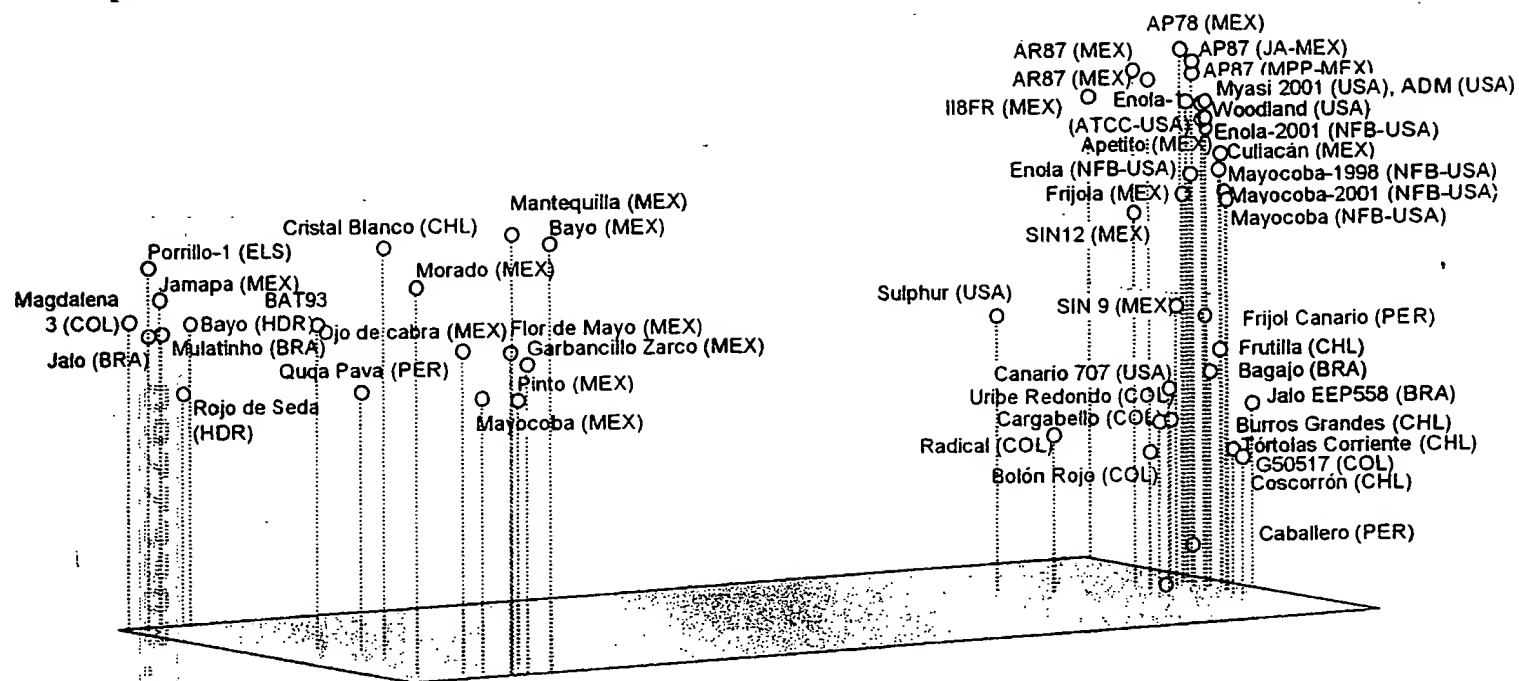   Bolón Rojo (COL)   Coscorrón (CHL)

Caballero (PER)

Figure 2. Relationships among bean cultivars detected by the AFLP technique as analyzed by Principal Coordinate Analysis. Cultivars colored in yellow have seeds with a yellow color similar to that of Enola seeds. Cultivars contained in the ellipse belong specifically to the Peruano commercial class.
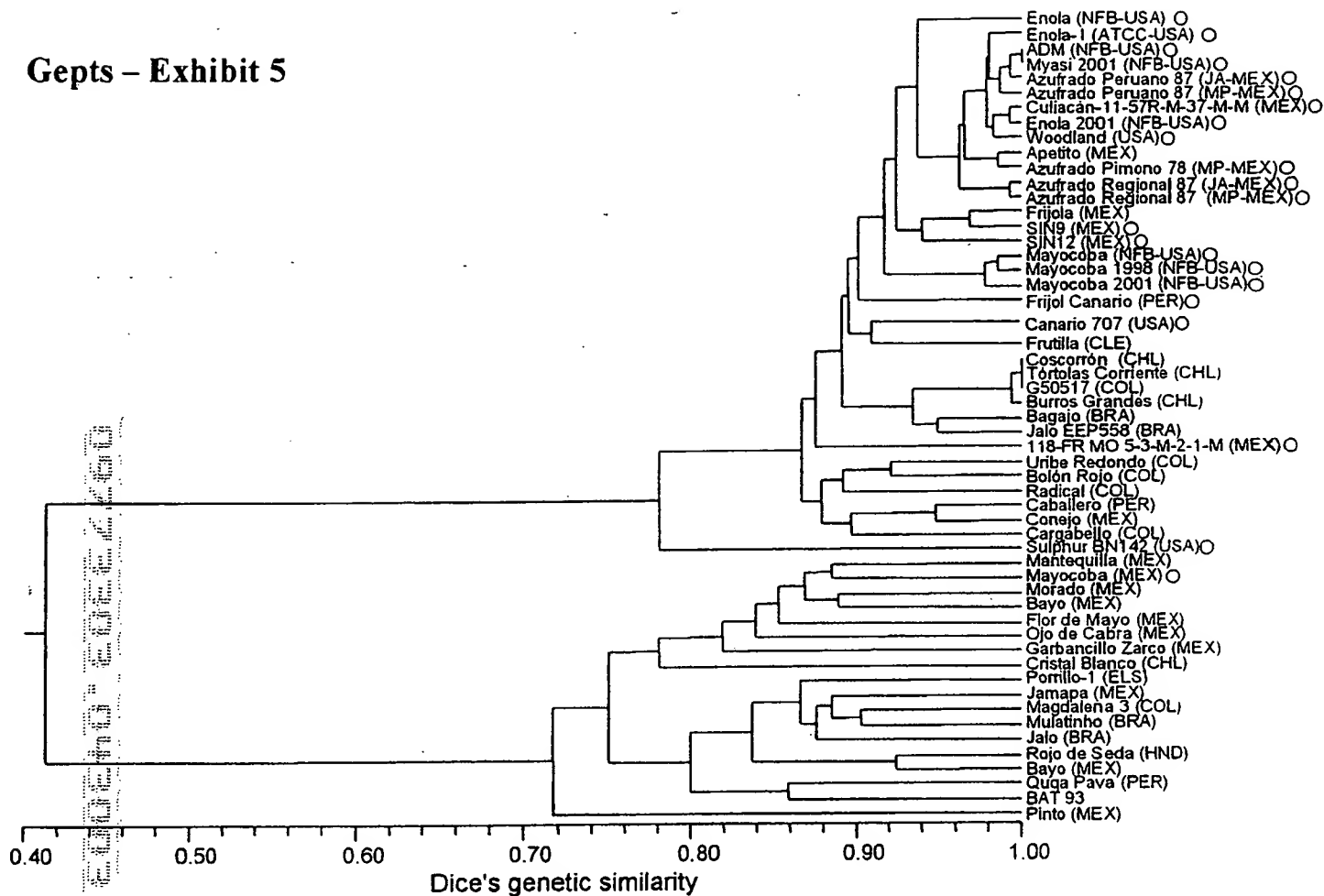
Exhibit 5

Gepts – Exhibit 5

Figure 3. A dendrogram showing relationships among yellow-seeded cultivars (followed by a yellow circle). Enola (obtained from ATCC) is located second from the top. The Mexican material most closely related to Enola is Azufrado Peruano 87.

Exhibit 6

AP78 (MEX)

AP87 (JA-MEX)

AR87 (MEX)

AP87 (MP-MEX)

AR87 (MEX)

Myasi-2001 (USA), ADM (USA)

Enola-1
(ATCC-USA)

II8FR (MEX)

Woodland Yellow (USA)

Apetito (MEX)

Enola-2001 (NFB-USA)

Culiacán (MEX)

Enola (NFB-USA)

Mayocoba-1998 (NFB-USA)

Mayocoba-2001 (NFB-USA)

Frijola (MEX)

Mayocoba (NFB-USA)

SIN12 (MEX)
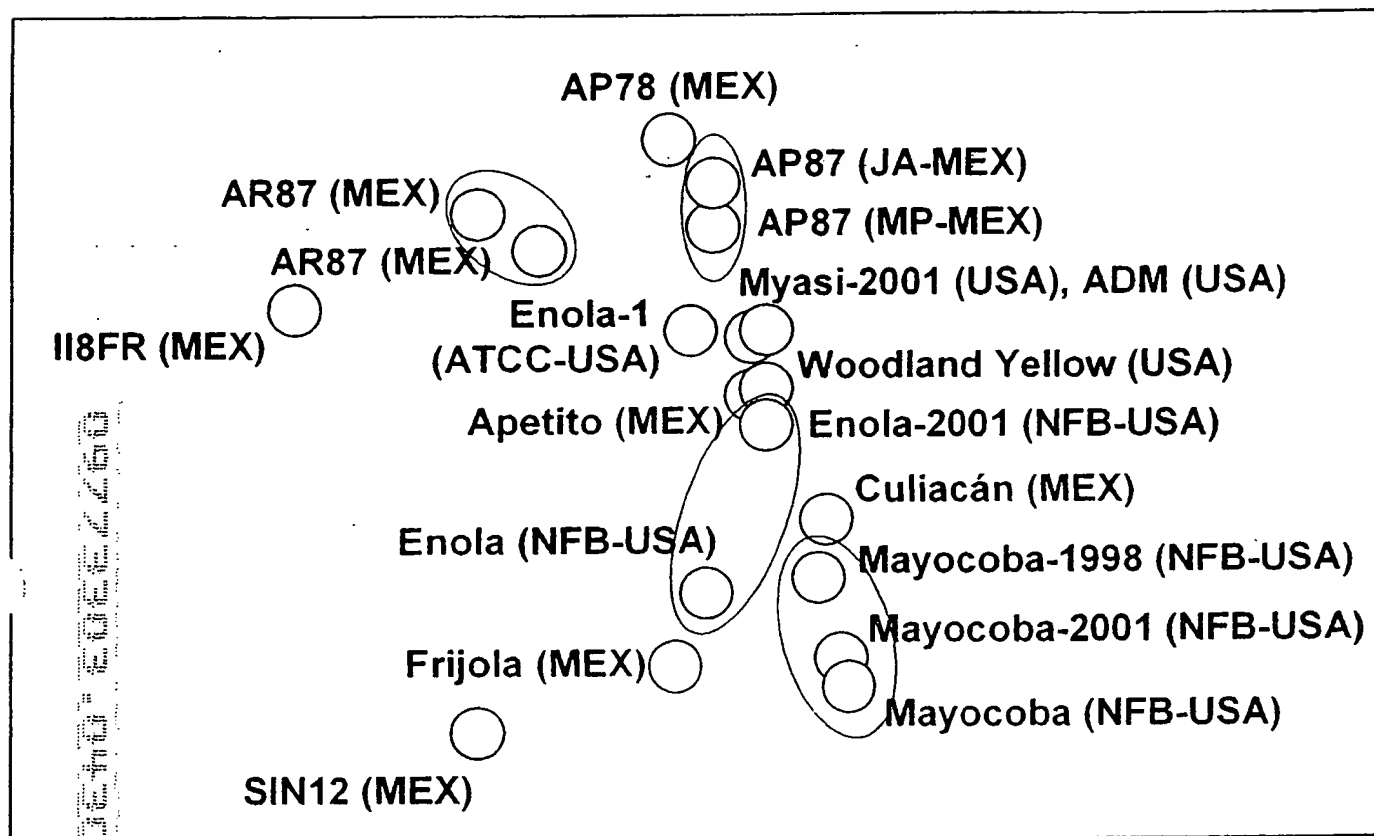
Figure 4. Close-up of Peruano cultivars from Figure 2. The distances among circles correspond to the genetic distances among individuals. The figure shows the close relationship of Enola with the cultivar Azufrado Peruano 87 (AP87) and the slight molecular differences among individuals of the same cultivar. Blue ellipses surround individuals taken from different samples of the same cultivar obtained from the same source.
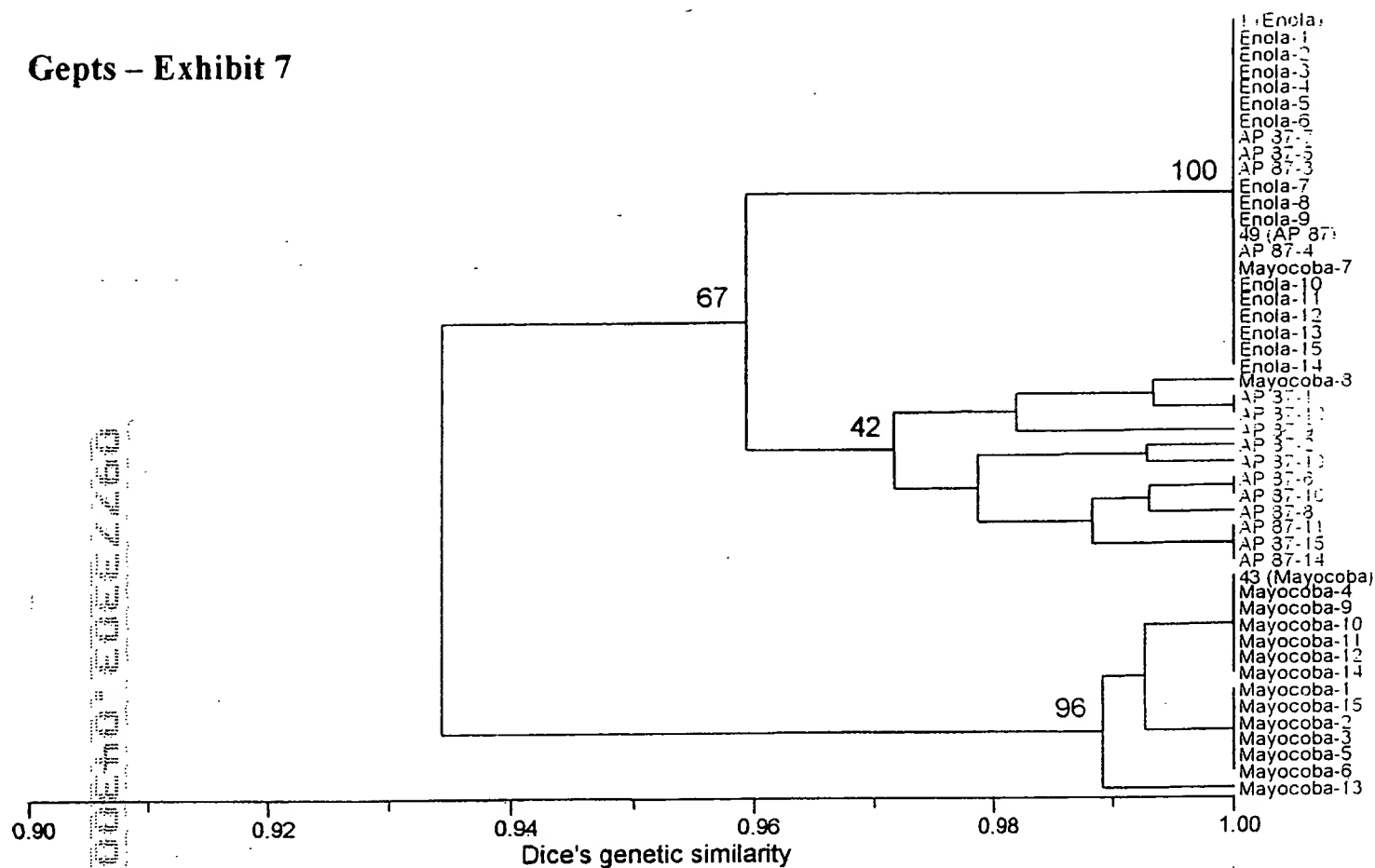
Exhibit 7

Gepts – Exhibit 7

Figure 5. Dendrogram showing the relationships among haplotypes (combination of molecular alleles) found in three Peruano-type cultivars: Enola (red color), Azufrado Peruano 87 (green color), and Mayocoba NF&B (blue color). Each branch represents a different haplotype. Individuals to the right of vertical bars have identical haplotypes. Of note here is the uniformity of Enola, which shows a single haplotype that is also found in the two other cultivars. The numbers within the tree are so-called bootstrap values (for explanations, see text).

Exhibit 8

Exhibit 8

Gepts – Exhibit 8

Table 2. Distribution of haplotypes among three Peruano-type cultivars

Cultivar (origin of sample)	Haplotype	Number of individuals in each haplotype	Number of haplotypes per sample	Total number of individuals analyzed per sample
Enola (ATCC)	A	16	1	16
Mayocoba (NF&B)	A	1		
	B	7		
	C	6		
	D	1		
	E	1	5	16
Azufrado Peruano (MPP)	A	5		
	F	2		
	G	1		
	H	2		
	I	1		
	J	1		
	K	3		
	L	1	8	16

CERTIFICATE OF SERVICE

I hereby certify that on October 7, 2002, a true and correct copy of the following document: **Expert Report of Paul Gepts** was served:

By First Class Mail on:

David J. Lee
Neil L. Arney
LATHROP & GAGE
4845 Pearl East Circle, Suite 302
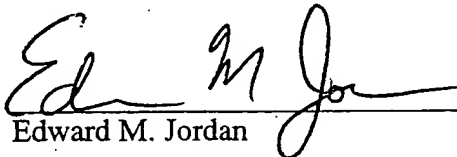Boulder, CO 80301

Ruth Quintanilla

40533869.1

CERTIFICATE OF SERVICE

I hereby certify that on October 31, 2002, a true and correct copy of the following

document: **EXPERT REPORT OF PAUL GEPTS PH.D. IN SUPPORT OF MOTION FOR**

SUMMARY ADJUDICATION THAT PLAINTIFF'S PLANT VARIETY PROTECTION

CERTIFICATE IS INVALID, OR IN THE ALTERNATIVE NOT INFRINGED was

served via Federal Express on:

Neil L. Arney
David Lee
LATHROP & GAGE
4845 Pearl East Circle, Suite 302
Boulder, CO 80301

Edward M. Jordan

40558862.1

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DEPARTMENT OF AGRONOMY AND RANGE SCIENCE
UNIVERSITY OF CALIFORNIA
1 SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8515
http://agronomy.ucdavis.edu/gepts/geptslab.htm

Phone: (530) 752-7743
FAX: (530) 752-4361
plgepts@ucdavis.edu

October 21, 2002

Ms. J. PIETRINI, Esq
Manatt, Phelps, and Phillips, LLP
11355 West Olympic Boulevard
Los Angeles, CA 90064-1614

Dear Ms. Pietrini,

Per your request, I am sending you the data sets for experiments 1 and 2 conducted in my laboratory in relation to the analyses of yellow-seeded bean cultivars.

For experiment 1 data, the yellow-colored columns correspond to yellow-seed entries in the experiment. For the identity of the materials, one should consult Exhibit 2, Table 1 of my report. AFLP fragments scored in experiment 1 are labeled consecutively from 1 to 151 in the leftmost column.

For experiment 2 data, the three colors correspond to the three varieties tested (Enola ATCC, Mayocoba NF&B, and Azufrado Peruano 87). The color coding is the same as in Exhibit 7, Figure 5 of my report. The leftmost column includes the consecutive numbering (from 1 to 133) of the AFLP fragments scored in this experiment. For both experiments, a value of 1 indicates the presence of the fragment and that of a 0 its absence in an entry.

Please feel free to contact me for additional information if necessary.

Yours truly,

Paul Gepts
Professor of Agronomy

B

Experiment 1

Individuals Analyzed (for meaning of labels, see Exhibit 2 Table 1)

AFLP

lines - Yellow
are on
originals

yellow
yellow
yellow
yellow
yellow

146 0 0 0 1 ...
147 1 1 0 1 ...
148 0 0 0 1 ...
149 0 0 0 1 ...
150 1 1 0 1 ...
151 1 0 0 1 ...

yellow

Individuals Analyzed

En08 | Mayocoba NF8B | Azufrado Pensano 87

Column headers (left to right):
AFLP, E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13, E14, E15, E16, E17, E18, E19, 43, G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, 49, F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15

Row labels (AFLP): 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33

AFLP: a new technique for DNA fingerprinting

Pieter Vos*, Rene Hogers, Marjo Bleeker, Martin Reijans, Theo van de Lee, Miranda Hornes, Adrie Frijters, Jerina Pot, Johan Peleman, Martin Kuiper and Marc Zabeau

Keygene N.V., PO Box 216, Wageningen, The Netherlands

ABSTRACT

A novel DNA fingerprinting technique called AFLP is described. The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. Typically 50–100 restriction fragments are amplified and detected on denaturing polyacrylamide gels. The AFLP technique provides a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity.

INTRODUCTION

DNA fingerprinting involves the display of a set of DNA fragments from a specific DNA sample. A variety of DNA fingerprinting techniques is presently available (1–11), most of which use PCR for detection of fragments. The choice of which fingerprinting technique to use, is dependent on the application e.g. DNA typing, DNA marker mapping and the organism under investigation e.g. prokaryotes, plants, animals, humans. Ideally, a fingerprinting technique should require no prior investments in terms of sequence analysis, primer synthesis or characterization of DNA probes. A number of fingerprinting methods which meet these requirements have been developed over the past few years, including random amplified polymorphic DNA (RAPD; 8), DNA amplification fingerprinting (DAF; 9) and arbitrarily primed PCR (AP-PCR; 10,11). These methods are all based on the amplification of random genomic DNA fragments by arbitrarily selected

PCR primers. DNA fragment patterns may be generated of any DNA without prior sequence knowledge. The patterns generated depend on the sequence of the PCR primers and the nature of the template DNA. PCR is performed at low annealing temperatures to allow the primers to anneal to multiple loci on the DNA. DNA fragments are generated when primer binding sites are within a distance that allows amplification. In principle, a single primer is sufficient for generating band patterns. These new PCR based fingerprinting methods have the major disadvantage that they are very sensitive to the reaction conditions, DNA quality and PCR temperature profiles (12–16), which limits their application.

This paper describes a new technique for DNA fingerprinting, named AFLP. The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNAs of any origin or complexity. Fingerprints are produced without prior sequence knowledge using a limited set of generic primers. The number of fragments detected in a single reaction can be 'tuned' by selection of specific primer sets. The AFLP technique is robust and reliable because stringent reaction conditions are used for primer annealing; the reliability of the RFLP technique (17,18) is combined with the power of the PCR technique (19–21). This paper describes several features of the AFLP technique and illustrates how the technique can best be used in fingerprinting of genomic DNAs.

MATERIALS AND METHODS

DNAs, enzymes and materials

Lambda DNA was purchased from Pharmacia (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). *Autographa californica* Nuclear Polyhedrosis Virus DNA (AcNPV) was a kind gift from Dr Just Vlak, Department of Virology, Agricultural University of Wageningen, The Netherlands, and was isolated as described previously (22). Acinetobacter DNA was a kind gift from Dr Paul Jansen, Department of Microbiology, University of Gent, Belgium, and was isolated from strain LMG 10554 according to the procedure of Pitcher *et al.* (23). Yeast DNA was isolated from strain AB1380 as described by Green and Olson with minor modifications (24). Tomato DNA (culture variety [cv] Moneymaker, obtained from Dr Maarten Koornneef, University of Wageningen, The Netherlands), Arabidopsis DNA (Recombinant Inbred Line 240, obtained from Dr Caroline Dean, John Innes Center, Norwich, UK), maize DNA (strain B73, obtained from Dr Mario Motto, Instituto Sperimentale per La, Bergamo, Italy), cucumber DNA (cv Primera, obtained from De Ruiter

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*To whom correspondence should be addressed

Seeds C.V., Bleiswijk, The Netherlands), barley DNA (cv Ingrid, obtained from Dr Paul Schulze-Lefert, University of Aachen, Germany), lettuce DNA (cv Calmar, obtained from Dr Richard Michelmore, UC Davis, Davis, CA, USA) and brassica DNA (oil seed rape, cv Major, obtained from Dr Thomas Osborn, University of Wisconsin, Madison, WI, USA) were isolated using a modified CTAB procedure described by Stewart and Via (25). Human DNA was prepared as described by Miller *et al.* (26) from a 100 ml blood sample of Mrs Marjo Bleeker, one of the co-authors of this paper. All restriction enzymes were purchased from Pharmacia (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), except for the restriction enzyme *MseI*, which was purchased from New England Biolabs Inc. (Beverly, MA, USA). T4 DNA ligase and T4 polynucleotide kinase were also obtained from Pharmacia (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). All PCR reagents and consumables were obtained from Perkin Elmer Corp. (Norwalk, CT, USA). All radioactive reagents were purchased from Amersham (Amersham International plc, Little Chalfont, Buckinghamshire, UK) or Isotopchim (Isotopchim SA, Ganagobie, France).

AFLP primers and adapters

All oligonucleotides were made on a Biotronic Synostat D DNA-synthesizer (Eppendorf Gmbh. Maintal, Germany) or Milligen Expedite 8909 DNA-synthesizer (Millipore Corp. Bedford, MA, USA). The quality of the crude oligonucleotides was determined by end-labeling with polynucleotide kinase and $[\gamma^{-32}P]ATP$ and subsequent electrophoresis on 18% denaturing polyacrylamide gels (27). Oligonucleotides were generally used as adapters and primers for AFLP analysis without further purification.

AFLP adapters consist of a core sequence and an enzyme-specific sequence (28). The structure of the *EcoRI*-adapter is:

```
5-CTCGTAGACTGCGTACC
  CATCTGACGCATGGTTAA-5
```

The structure of the *MseI*-adapter is:

```
5-GACGATGAGTCCTGAG
  TACTCAGGACTCAT-5
```

Adapters for other 'rare cutter' enzymes were identical to the *EcoRI*-adapter with the exception that cohesive ends were used, which are compatible with these other enzymes. The *TaqI*-adapter was identical to the *MseI*-adapter with the exception that a cohesive end was used compatible with *TaqI*.

AFLP primers consist of three parts, a core sequence, an enzyme specific sequence (ENZ) and a selective extension (EXT) (28). This is illustrated below for *EcoRI*- and *MseI*-primers with three selective nucleotides (selective nucleotides shown as NNN):

```
           CORE        ENZ    EXT
EcoRI   5-GACTGCGTACC AATTC NNN-3
MseI    5-GATGAGTCCTGAG TAA NNN-3
```

AFLP-primers for other 'rare cutter' enzymes were similar to the *EcoRI*-primers, and *TaqI*-primers were similar to the *MseI*-primers, but have enzyme-specific parts corresponding to the respective enzymes.

Modification of DNA and template preparation

The protocol below describes the generation of templates for AFLP reactions using the enzyme combination *EcoRI/MseI*. DNA templates with other restriction enzymes were prepared using essentially the same protocol, except for the use of different restriction enzymes and corresponding double-stranded adapters.

Genomic DNA (0.5 μg) was incubated for 1 h at 37°C with 5 U *EcoRI* and 5 U *MseI* in 40 μl 10 mM Tris–HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/μl BSA. Next, 10 μl of a solution containing 5 pMol *EcoRI*-adapters, 50 pMol *MseI*-adapters, 1 U T4 DNA-ligase, 1 mM ATP in 10 mM Tris–HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/μl BSA was added, and the incubation was continued for 3 h at 37°C. Adapters were prepared by adding equimolar amounts of both strands; adapters were not phosphorylated. After ligation, the reaction mixture was diluted to 500 μl with 10 mM Tris–HCl, 0.1 mM EDTA pH 8.0, and stored at –20°C.

AFLP reactions

Amplification reactions are described using DNA templates for the enzyme combination *EcoRI/MseI*. AFLP fingerprints with other enzyme combinations were performed with appropriate primers.

AFLP reactions generally employed two oligonucleotide primers, one corresponding to the *EcoRI*-ends and one corresponding to the *MseI*-ends. One of two primers was radioactively labeled, preferably the *EcoRI*-primer. The primers were end-labeled using $[\gamma^{-33}P]ATP$ and T4 polynucleotide kinase. The labeling reactions were performed in 50 μl 25 mM Tris–HCl pH 7.5, 10 mM MgCl₂, 5 mM DTT, 0.5 mM spermidine–3HCl using 500 ng oligonucleotide primer, 100 μCi $[\gamma^{-33}P]ATP$ and 10 U T4 polynucleotide kinase. Twenty μl PCRs were performed containing 5 ng labeled *EcoRI*-primer (0.5 μl from the labeling reaction mixture), 30 ng *MseI*-primer, 5 μl template-DNA, 0.4 U *Taq* polymerase, 10 mM Tris–HCl pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.2 mM of all four dNTPs.

The PCR conditions differed depending on the nature of the selective extensions of the AFLP primers used for amplification. AFLP reactions with primers having none or a single selective nucleotide were performed for 20 cycles with the following cycle profile: a 30 s DNA denaturation step at 94°C, a 1 min annealing step at 56°C, and a 1 min extension step at 72°C. AFLP reactions with primers having two or three selective nucleotides were performed for 36 cycles with the following cycle profile: a 30 s DNA denaturation step at 94°C, a 30 s annealing step (see below), and a 1 min extension step at 72°C. The annealing temperature in the first cycle was 65°C, was subsequently reduced each cyle by 0.7°C for the next 12 cycles, and was continued at 56°C for the remaining 23 cycles. All amplification reactions were performed in a PE-9600 thermocycler (Perkin Elmer Corp., Norwalk, CT, USA).

AFLP fingerprinting of complex genomes generally involved an amplification in two steps. The first step of this amplification procedure, named preamplification, was performed with two AFLP primers having a single selective nucleotide as described above, with the exception that 30 ng of both AFLP primers was used, and that these primers were not radioactively labeled. After this preamplification step, the reaction mixtures were diluted 10-fold with 10 mM Tris–HCl, 0.1 mM EDTA pH 8.0, and used
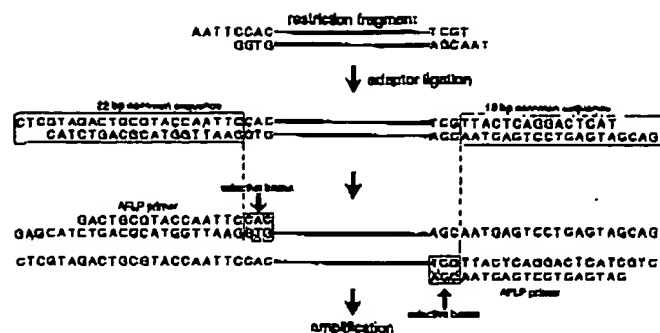
Figure 1. Schematic representation of the AFLP technique. Top: EcoRI–MseI restriction fragment with its 5' protruding ends. Center: the same fragment after ligation of the EcoRI and MseI adaptors. Bottom: both strands of the fragment with their corresponding AFLP primers. The 3' end of the primers and their recognition sequence in the EcoRI–MseI fragment are highlighted.

as templates for the second amplification reaction. The second amplification reaction was performed as described above for AFLP reactions with primers having longer selective extensions.

Gel analysis

Following amplification reaction products were mixed with an equal volume (20 µl) of formamide dye (98% formamide, 10 mM EDTA pH 8.0. and bromo phenol blue and xylene cyanol as tracking dyes). The resulting mixtures were heated for 3 min at 90°C, and then quickly cooled on ice. Each sample (2 µl) was loaded on a 5% denaturing (sequencing) polyacrylamide gel (27). The gel matrix was prepared using 5% acrylamide, 0.25% methylene bisacryl, 7.5 M urea in 50 mM Tris/50 mM Boric acid/1 mM EDTA. To 100 ml of gel solution 500 µl of 10% APS and 100 µl TEMED was added and gels were cast using a SequiGen 38 × 50 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). 100 mM Tris/100 mM Boric acid/2 mM EDTA was used as running buffer. Electrophoresis was performed at constant power, 110 W, for ~2 h. After electrophoresis, gels were fixed for 30 min in 10% acetic acid dried on the glass plates and exposed to Fuji phosphoimage screens for 16 h. Fingerprint patterns were visualized using a Fuji BAS-2000 phosphoimage analysis system (Fuji Photo Film Company Ltd, Japan).

RESULTS AND DISCUSSION

Principle of the method

The AFLP technique is based on the amplification of subsets of genomic restriction fragments using PCR. DNA is cut with restriction enzymes, and double-stranded (ds) adapters are ligated to the ends of the DNA-fragments to generate template DNA for amplification. The sequence of the adapters and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments (Fig. 1). Selective nucleotides are included at the 3' ends of the PCR primers, which therefore can only prime DNA synthesis from a subset of the restriction sites. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified (Fig. 1).

The restriction fragments for amplification are generated by two restriction enzymes, a rare cutter and a frequent cutter. The AFLP
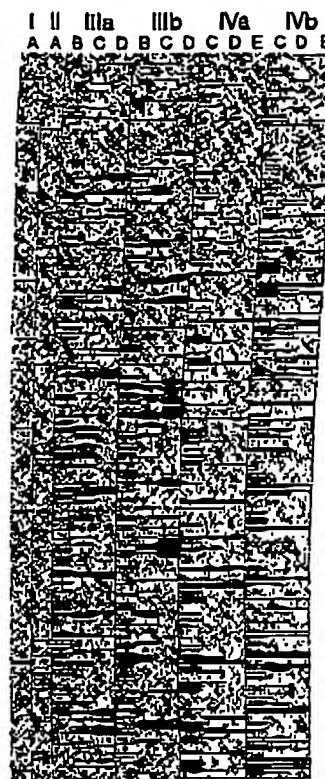

Figure 2. AFLP fingerprints of genomic DNAs of various complexities: λ DNA (panel I), AcNPV DNA (panel II), Acinetobacter DNA (panels IIIa and IIIb) and yeast DNA (panels IVa and IVb). Letters A, B, C, D and E refer to none, one, two, three and four selective bases in the AFLP primers respectively. The primer combinations used were from left to right: I. EcoRI+0/MseI+0, II. EcoRI+0/MseI+0, IIIa. EcoRI+0/MseI+A, EcoRI+C/MseI+A, EcoRI+C/MseI+AT. IIIb. EcoRI+0/MseI+T, EcoRI+C/MseI+T, EcoRI+C/MseI+TA, IVa. EcoRI+C/MseI+G, EcoRI+C/MseI+GC, EcoRI+CA/MseI+GC, IVb. EcoRI+C/MseI+T, EcoRI+C/MseI+TA, EcoRI+CA/MseI+TA. (+0 indicates no selective nucleotides, +A indicates selective nucleotide = A, etc). The molecular weight size range of the fingerprints is 45–500 nucleotides.

procedure results in predominant amplification of those restriction fragments, which have a rare cutter sequence on one end and a frequent cutter sequence on the other end (this will be explained below, see also Fig. 3). The rationale for using two restriction enzymes is the following. (i) The frequent cutter will generate small DNA fragments, which will amplify well and are in the optimal size range for separation on denaturing gels (sequence gels). (ii) The number of fragments to be amplified is reduced by using the rare cutter, since only the rare cutter/frequent cutter fragments are amplified. This limits the number of selective nucleotides needed for selective amplification. (iii) The use of two restriction enzymes makes it possible to label one strand of the ds PCR products, which prevents the occurence of 'doublets' on the gels due to unequal mobility of the two strands of the amplified fragments. (iv) Using two different restriction enzymes gives the greatest flexibility in 'tuning' the number of fragments to be amplified. (v) Large numbers of different fingerprints can be generated by the various combinations of a low number of primers.

AFLP fingerprinting of simple genomes

The AFLP technique makes use of ds adapters ligated to the ends of the restriction fragments to create target sites for primer
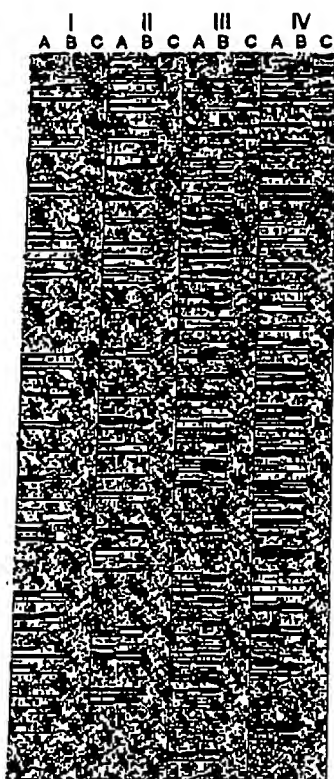
Figure 3. Illustration of the principle of preferential amplification of EcoRI–MseI fragments. AFLP fingerprints are shown of yeast DNA using primer combinations with a single selective base for the EcoRI primers and two selective bases for the MseI primer. Panels I, II, III and IV refer to the primer combinations EcoRI+C/MseI+AC, EcoRI+C/MseI+CA, EcoRI+T/MseI+AG and EcoRI+T/MseI+AT. respectively. Lanes A and B show standard AFLP fingerprints with either the EcoRI primers labeled (lanes A) or the MseI primers labeled (lanes B). In lanes C the MseI primers were labeled but the EcoRI primers were omitted from the AFLP reaction. The molecular weight size range of the fingerprints is from 80 to 500 nucleotides.

annealing in fragment amplification. A subset of the restriction fragments is specifically amplified by the use of selective nucleotides at the 3′ ends of the AFLP primers. This principle was demonstrated by fingerprinting of four different simple genomic DNAs, varying in genome size from 48.5 to 16 000 kb. The following genomic DNAs were selected and fingerprinted using the enzyme combination EcoRI/MseI: phage λ DNA (48 451 bp) (29), AcNPV DNA (*Autographa californica* Nuclear Polyhedrosis Virus, 129 981 bp) (30), Acinetobacter DNA (estimated genome size 3000 kb) (31) and yeast DNA (estimated genome size 16 000 kb) (32) (Fig. 2).

For the DNAs of phage λ and AcNPV, the complete nucleotide sequence is known, and therefore all EcoRI/MseI fragments could be exactly predicted. Indeed, all predicted fragments were detected. Also for Acinetobacter and yeast DNA, the number of fragments corresponded well with the genome sizes of these organisms. Adding selective nucleotides to the AFLP primers reduced the number of bands ~4-fold with each additional selective base. Furthermore, the addition of an extra selective nucleotide always resulted in a fingerprint, which was a subset of the original fingerprint. This indicates that the selective nucleotides are an accurate and efficient way to select a specific set of restriction

fragments for amplification. The AFLP fingerprints showed that large numbers of restriction fragments were amplified simultaneously, and that in principle the number of bands detected is limited only by the power of the detection system, i.e. polyacrylamide gels. In general, the simultaneous amplification of DNA fragments using specific primer sets for each PCR fragment (multiplex PCR) appears to be rather troublesome (33–35). Our results suggest that multi-fragment amplification is efficient provided that all fragments use the same primer set for their amplification. This implies that the differences in amplification efficiency of DNA fragments in PCR (33–35), are mainly primer-associated and not fragment specific.

The ds adapters used for ligation to the restriction fragments are not phosphorylated, which causes only one strand to be ligated to the ends of the restriction fragments. Fragments were therefore not amplified if the template DNAs were denatured prior to PCR amplification (results not shown), except when *Taq* polymerase and dNTPs were present before denaturation. The filling-in of the 3′ recessed ends by the *Taq* polymerase following the dissociation of the non-ligated strands during the heating step seems a matter of only seconds or less. Alternatively, the *Taq* polymerase may immediately displace the non-ligated strands at low temperatures in the process of assembling the reaction mixtures.

These results demonstrated that: (i) the AFLP technique provides an efficient way to amplify large numbers of fragments simultaneously, (ii) the amplified fragments are restriction fragments, (iii) the number of fragments obtained increased as the genome size increased and corresponded well with what was theoretically expected, (iv) the selective nucleotides at the ends of the AFLP primers reduced the number of bands precisely as would be expected.

Restriction of the genomic DNA with EcoRI and MseI will result in three classes of restriction fragments, MseI–MseI fragments, EcoRI–MseI fragments and EcoRI–EcoRI fragments. The vast majority (>90%) are expected to be MseI–MseI fragments, the EcoRI–MseI fragments will be about twice the number of EcoRI restriction sites and a small number of the fragments will be EcoRI–EcoRI fragments. In the previous experiments the EcoRI primer was labeled and, therefore, only the restriction fragments with an EcoRI site could be detected. To investigate the amplification of the MseI–MseI fragments a set of AFLP reactions on yeast DNA was performed with the MseI primer labeled instead of the EcoRI primer (Fig. 3): this is expected to show the EcoRI–MseI fragments as well as the MseI–MseI fragments.

Similar yeast fingerprints were obtained with either the EcoRI or MseI primer labeled (Fig. 3, compare lanes A and B). However, most of the bands showed a significant shift in mobility, due to the fact that the other strand of the fragments was detected. It was surprising to find that almost no additional fragments, i.e. MseI–MseI fragments. were detected upon labeling of the MseI primer in stead of the EcoRI primer. Therefore, additional reactions were performed to which only the MseI primer was added, which will theoretically only allow amplification of MseI–MseI fragments (Fig. 3. C lanes). Indeed, these reactions showed amplification products not observed in the presence of the EcoRI primers. These observations imply that amplification of the MseI–MseI fragments is inefficient in the presence of the EcoRI primer, i.e. that there is preferential amplification of EcoRI–MseI fragments compared with the MseI–MseI fragments in the AFLP reaction. This may be explained in two ways. (i) The

Figure 4. Illustration of the selectivity of AFLP primers. AFLP fingerprints are shown of yeast DNA using primer combinations with one selective base for the *Eco*RI primer and one (lanes A), two (lanes B), three (lanes C) and four (lanes D) selective nucleotides respectively for the *Mse*I primer. The primer combinations used are *Eco*RI+A/*Mse*I+CTCA (panel I), *Eco*RI+A/*Mse*I+CTGC (panel II) and *Eco*RI+T/*Mse*I+CTCA (panel III). The molecular weight size range of the fingerprints is from 40 to 370 nucleotides.

*Mse*I primer has a lower annealing temperature than the *Eco*RI primer, making amplification of *Mse*I–*Mse*I fragments less efficient compared with *Eco*RI–*Mse*I fragments under the conditions used. We have indeed observed stronger amplification of *Mse*I–*Mse*I fragments using longer or alternative *Mse*I primers and adapters. (ii) The *Mse*I–*Mse*I fragments have an inverted repeat at the ends, due to the fact that they are amplified by a single primer. Therefore, a stem–loop structure may be formed by base-pairing of the ends of the fragments, which will compete with primer annealing. This is also confirmed by the observation that only larger fragments were amplified in AFLP reactions when only the *Mse*I primer was added. Formation of a stem–loop structure will be more difficult in these larger fragments. We have indeed demonstrated that amplification of large fragments (>1 kb) with a single primer is quite efficient (results not shown).

Careful primer design is crucial for succesful PCR amplification. AFLP primers consist of three parts: the 5′ part corresponding to the adapter, the restriction site sequence and the 3′ selective nucleotides. Therefore, the design of AFLP primers is mainly determined by the design of the adapters, which are ligated to the restriction fragments. Various adapter designs were tested (results not shown), all with good results provided that the general rules for 'good PCR primer design' were followed (36,37). A different adapter design will demand different PCR

conditions, and it is therefore important to select a specific design for the adapters, and to optimize the conditions for this design. An important feature of the AFLP primers is that all primers start with a 5′ guanine (G) residue. We have found that a 5′ G-residue in the unlabeled primer is crucial to prevent the phenomenon of double bands. The double bands appeared to result from incomplete addition of an extra nucleotide to the synthetised strands (results not shown). This terminal transferase activity of the *Taq* polymerase is quite strong, and has been frequently reported (38–40). We have also found that the 3′ nucleotide additions were influenced by the concentration of dNTPs, and that double bands occur at low concentrations of dNTPs regardless of the 5′-residue in the PCR primers (results not shown). We conclude from our results that this terminal transferase activity is most strong (almost 100% of the synthesized strands) when the 3′ nucleotide of the synthesized strand is a cytidine (C).

In the experiments described so far, AFLP primers were used with one or two selective 3′ nucleotides. This low number of selective nucleotides was shown to provide an effective way to select the desired number of fragments for amplification. Next, primers with longer 3′ extensions were tested to determine the maximum number of 3′ selective nucleotides which would retain high selectivity in AFLP reactions. For this purpose, fingerprints were generated of yeast DNA using primers with up to four selective nucleotides. A single *Eco*RI primer was selected with one selective nucleotide and combined with four different *Mse*I primers with one, two, three or four selective nucleotides, respectively. The *Mse*I selective extensions were choosen in a way that with each additional selective nucleotide a fingerprint would be generated that is the subset of the preceding fingerprints, e.g. extensions +C, +CT, +CTC, +CTCA. The appearance of bands that do not occur in the preceding fingerprints is an indication that fragments are amplified which do not correspond to the sequence of the selective bases, and consequently that selectivity is incomplete (Fig. 4).

The fingerprints shown in Figure 4 and the fingerprints from previous experiments demonstrated that primer selectivity is good for primers with one or two selective nucleotides. Selectivity is still acceptable with primers having three selective nucleotides, but it is lost with the addition of the fourth nucleotide. The loss of selectivity with primers having four selective bases is illustrated by the amplification of numerous bands not detected in the corresponding fingerprints with primers having three selective bases (compare lanes D with lanes C). This indicates the tolerance of mismatches in the amplification of the fragments using AFLP primers with four-base extensions. It is most likely that mismatches will be tolerated at the first selective base, because this nucleotide is positioned most distant from the 3′ end, and because the selectivity of three-base selective extensions was still adequate. Other researchers have also investigated the selectivity of the 3′ nucleotides of PCR primers (41,42). They have found that mismatches were tolerated at both the 3′ ultimate and penultimate nucleotides of PCR primers, which is in conflict with our findings. In other experiments, we have found that primer selectivity is relative and also depends on the number of fragments amplified in a single reaction, the PCR conditions and the primer design (results not shown). We feel that some level of mismatch amplification will always occur, but that the reaction conditions can prevent these mismatch bands to reach the

detection level. This, presumably, is the major difference with previously reported experiments (41,42).

AFLP fingerprinting of complex genomes

Initial experiments with AFLP fingerprinting of a number of plant and animal DNAs indicated that AFLP primers with at least three selective nucleotides at both the *Eco*RI and *Mse*I primer were required to generate useful band patterns. Because primers with three selective bases tolerate a low level of mismatch amplification, a two-step amplification strategy was developed for AFLP fingerprinting of complex DNAs. In the first step, named preamplification, the genomic DNAs were amplified with AFLP primers both having a single selective nucleotide. Next the PCR products of the preamplification reaction were diluted and used as template for the second AFLP reaction using primers both having three selective nucleotides. We have compared this amplification strategy with a direct amplification of complex genomic DNAs without the use of the preamplification step. The two-step amplification strategy resulted in two important differences compared with the direct AFLP amplification: (i) background 'smears' in the fingerprint patterns were reduced, and (ii) fingerprints with particular primer combinations lacked one or more bands compared with fingerprints generated without preamplification. This is best explained assuming that the direct amplification with AFLP primers having three selective nucleotides resulted in a low level of mismatch amplification products, which caused the background smears and gave discrete amplified fragments corresponding to repeated restriction fragments. An additional advantage of the two-step amplification strategy is that it provides a virtually unlimited amount of template DNA for AFLP reactions. Figure 5 shows a number of typical AFLP fingerprints obtained with the two-step amplification strategy using DNAs of three plant species, *Arabidopsis thaliana*, tomato (*Lycopersicon esculentum*) and maize (*Zea mays*), and human DNA. For Arabidopsis DNA primer combinations with a total of five selective nucleotides were used, because of the small genome of this plant species (145 Mb, 43). For tomato, maize and human DNA, six selective nucleotides were used, three selective bases for both the *Eco*RI and *Mse*I-primer.

 DNA fingerprinting methods based on amplification of genomic DNA fragments by random primers have been found to be quite susceptible to the template DNA concentration (13,14). DNA quantities may vary considerably between individual samples isolated by standard DNA isolation procedures. Preferably, a DNA fingerprinting technique should be insensitive to variations in DNA template concentration. Therefore, the sensitivity of the AFLP technique for the template DNA concentrations was investigated.

AFLP fingerprints were performed using tomato DNA and the enzyme combination *Eco*RI/*Mse*I. Template DNA quantities were varied from 2.5 pg to 25 ng. The standard two-step amplification protocol was used, with the exception of an extended preamplification step for the 2.5 and 25 pg DNA template samples. In tomato 2.5 pg of template DNA corresponds to approximately four molecules of each DNA fragment at the start of the AFLP reaction. AFLP fingerprint patterns were very similar using template quantities that ranged 1000-fold, i.e. from 25 ng to 25 pg (Fig. 6). Fingerprints generated with only 2.5 pg of template DNA were similar to the other fingerprints, although bands varied significantly in intensity and some bands were absent.

Figure 5. AFLP fingerprints of plant DNAs and human DNAs. Each panel shows three *Eco*RI–*Mse*I fingerprints using three different primer combinations. Human DNA fingerprints are displayed in panel IV, plant fingerprints are displayed in panels I (Arabidopsis), II (tomato) and III (maize), respectively. Primer combinations are from left to right 1. *Eco*RI+CA/*Mse*I+CTT. 2. *Eco*RI+CA/*Mse*I+CAT. 3. *Eco*RI+CA/*Mse*I+CTC, 4. *Eco*RI+ACC/*Mse*I+CTT. 5. *Eco*RI+ACC/*Mse*I+CTC, 6. *Eco*RI+ACC/*Mse*I+CTA, 7. *Eco*RI+ACC/*Mse*I+CAT, 8. *Eco*RI+AGG/*Mse*I+CTT, 9. *Eco*RI+AGG/*Mse*I+CAA, 10. *Eco*RI+CAC/*Mse*I+CGA, 11. *Eco*RI+CAC/*Mse*I+CAA, 12. *Eco*RI+CAG/*Mse*I+CGA. The molecular weight size range of the fingerprints is from 45 to 500 nucleotides.

These results demonstrate that the AFLP procedure is insensitive to the template DNA concentration, although aberrant fingerprints will be observed at very high template dilutions giving only a few template molecules at the start of the reaction. Most probably the individual restriction fragments are not randomly distributed at such low DNA concentrations explaining the observed differences in band intensities. This hypothesis is supported by our finding that comparison of a number of individual AFLP fingerprints obtained with only 2.5 pg of template DNA showed high variation in the intensity of the individual bands (results not shown).

A remarkable characteristic of the AFLP reaction is that generally the labeled primer is completely consumed (the unlabeled primer is in excess), and that therefore the amplification reaction stops when the labeled primer is exhausted. We have also found that further thermo cycling does not affect the band patterns once the labeled primer is consumed (results not shown). This characteristic is elegantly utilyzed in the AFLP protocol, which uses an excess of PCR cycles, which will result in fingerprints of equal intensity despite of variations in template concentration.

We have also noted that DNA fingerprints tend to be unreliable if the template concentration was below a certain absolute concentration (~1 pg), regardless of the complexity of the DNA
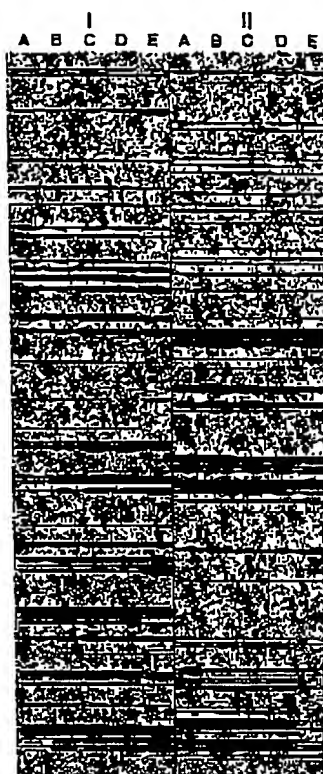
Figure 6. Illustration of the effect of template DNA concentration on AFLP fingerprinding. EcoRI–MseI fingerprints of tomato DNA are shown using two primer combinations EcoRI+ACC/MseI+CAT (panel I) and EcoRI+ACC/MseI+CTT (panel II). Five different DNA concentrations were used as AFLP templates: 25 ng (lanes A), 2.5 ng (lanes B), 250 pg (lanes C), 25 pg (lanes D) and 2.5 pg (lanes E). The molecular weight size range of the fingerprints is 40–370 nucleotides.

(results not shown). In the latter case, bands were observed which were template independent and which were also present if no DNA was added.

The use of other restriction enzyme combinations for AFLP fingerprinting of complex genomes was also investigated (results not shown). These enzyme combinations included EcoRI, HindIII, PstI, BglII, XbaI and Sse8387I (eight base cutter) in combination with either MseI or TaqI. Fingerprints were generated on a variety of plant and animal DNAs. The use of TaqI as a four-base cutter in stead of MseI resulted in an unequal distribution of the amplified fragments, which were mainly present in the upper part of the gel. Most eukaryotic DNAs are AT-rich, and as a result MseI (recognition sequence TTAA) will generally produce much smaller restriction fragments compared with TaqI (recognition sequence TCGA). MseI is therefore preferred for AFLP fingerprinting because it cuts very frequently in most eukaryotic genomes yielding fragments that are in the optimal size range for both PCR amplification and separation on denaturing polyacrylamide gels. Other rare cutter enzymes generally performed equal to EcoRI in AFLP fingerprinting. and the number of bands obtained reflected the cleavage frequency of the various restriction enzymes. However, EcoRI is preferred because it is a reliable (low cost) six-cutter enzyme, which limits problems associated with partial restriction in AFLP fingerprinting (see below).

Incomplete restriction of the DNA will cause problems in AFLP fingerprinting. because partial fragments will be generated. which will be detected by the AFLP procedure. When various DNA samples are compared with AFLP fingerprinting, incomplete restriction will result in the detection of differences in band patterns, which do not reflect true DNA polymorphisms, i.e. when one sample is partially restricted and the others are not. Incomplete restriction will only become apparent when different DNA samples of the same organism are compared. It is characterised by the presence of additional bands in the lanes, predominantly of higher molecular weight.

CONCLUSIONS

AFLP is a DNA fingerprinting technique that detects genomic restriction fragments and resembles in that respect the RFLP (restriction fragment length polymorphism) technique, with the major difference that PCR amplification instead of Southern hybridisation is used for detection of restriction fragments. The resemblance with the RFLP technique was the basis to chose the name AFLP. The name AFLP, however, should not be used as an acronym, because the technique will display presence or absence of restriction fragments rather than length differences.

In our initial AFLP protocols an additional purification step was incorporated in the template preparation (28). Biotinylated EcoRI-adapters were used for ligation, and subsequently the biotinylated DNA fragments (EcoRI–MseI fragments and EcoRI–EcoRI fragments) were substracted from the ligation mixture using streptavidin beads (28,44). This step reduced the complexity of the DNA template by removing all MseI–MseI fragments, which proved to be important for generating high quality fingerprints of complex DNAs. The protocol described in this paper omits this purification step, because amplification conditions are used which result in preferential amplification of EcoRI–MseI fragments with respect to the MseI–MseI fragments (Fig. 3). This is the result of careful adapter and primer design and inherent characteristics of the AFLP amplification reaction.

The AFLP technique will generate fingerprints of any DNA regardless of the origin or complexity, and in this paper we have presented AFLP fingerprints of DNAs differing in genome size as much as 100 000-fold. We have described that the number of amplified fragments may be controlled by the cleavage frequency of the rare cutter enzyme and the number of selective bases. In addition the number of amplified bands may be controlled by the nature of the selective bases; selective extensions with rare di- or trinucleotides will result in the reduction of amplified fragments. In general, there is an almost linear correlation between numbers of amplified fragments and genome size. This linear correlation is lost in the complex genomes of higher plants, which contain high numbers of repeated sequences and, hence, multicopy restriction fragments. Fingerprints of these complex DNAs consist predominantly of unique AFLP fragments, but are characterised by the presence of small numbers of more intense repeated fragments.

In complex genomes the number of restriction fragments that may be detected by the AFLP technique is virtually unlimited. A single enzyme combination (a combination of a specific six-base and four-base restriction enzyme) will already permit the amplification of 100 000s of unique AFLP fragments, of which generally 50–100 are selected for each AFLP reaction. Most AFLP fragments correspond to unique positions on the genome, and, hence, can be exploited as landmarks in genetic and physical

maps, each fragment being characterized by its size and its primers required for amplification. In addition, the AFLP technique permits detection of restriction fragments in any background or complexity, including pooled DNA samples and cloned (and pooled) DNA segments. Therefore, the AFLP technique is not simply a fingerprinting technique, it is an enabling technology in genome research, because it can bridge the gap between genetic and physical maps. First, the AFLP technique is a very effective tool to reveal restriction fragment polymorphisms. These fragment polymorphisms, i.e. AFLP markers, can be used to construct high density genetic maps of genomes or genome segments. In most organisms AFLP will prove to be the most effective way to construct genetic DNA marker maps compared to other existing marker technologies. Secondly, the AFLP markers can be used to detect corresponding genomic clones, e.g. yeast artificial chromosomes (YACs). This is most effectively achieved by working with libraries, which are pooled to allow for rapid PCR screening and subsequent clone identification. An AFLP marker will detect a single corresponding YAC clone in pools of as much as 100 YAC clones (M. Zabeau, M. Kuiper and P. Vos., manuscript in preparation). Finally, the AFLP technique may be used for fingerprinting of cloned DNA segments like cosmids, P1 clones, bacterial artificial chromosomes (BACs) or YACs (results not shown). By simply using no or few selective nucleotides, restriction fragment fingerprints will be produced, which subsequently can be used to line up individual clones and make contigs.

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Classical and Molecular Genetic Studies of the Strong Greenish Yellow Seedcoat Color in 'Wagenaar' and 'Enola' Common Bean

Mark J. Bassett[1]

Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Rian Lee,[2] Carla Otto,[3] and Phillip E. McClean[1]

Department of Plant Sciences, North Dakota State University, Fargo, ND 58105-5051

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ABSTRACT . Inheritance of the strong greenish-yellow (SGY) seedcoat color in 'Wagenaar' common bean (*Phaseolus vulgaris* L.) was investigated. Line 5-593 is a determinate, Florida dry bean breeding line (with small black seeds) used as the recurrent parent in the development of many genetic stocks, e.g., $g\ b\ v$ BC_3 5-593. Through crosses with genetic tester stocks, the seedcoat genotype of 'Wagenaar' was confirmed to be $C\ J\ g\ b\ v^{lae}\ Rk$. Three randomly amplified polymorphic DNA markers ($OAP7_{850}$, $OAP3_{1400}$, and $OU14_{950}$) that cosegregated with the G seedcoat color locus were developed from the F_2 population derived from the cross $g\ b\ v$ BC_2 5-593 x $G\ b\ v$ BC_3 5-593. From the cross 'Wagenaar' x $g\ b\ v$ BC_3 5-593, 80 F_2 plants were classified into 54 non-SGY and 16 SGY seedcoat color plants. When the $OAP7_{850}$ marker was applied to that population, linkage was not observed with the non-SGY and SGY phenotypes. Conversely, a molecular marker ($OAP12_{400}$, that was developed from the F_2 from the cross 'Wagenaar' x $g\ b\ v$ BC_3 5-593) linked to the locus controlling the SGY phenotype segregated independently of the G locus. Therefore, SGY phenotype is not controlled by the G locus. An F_3 progeny test of 76 F_2 plants from the cross 'Wagenaar' x $g\ b\ v$ BC_3 5-593 confirmed the hypothesis that a single recessive gene (for which we propose the symbol gy) controls the seedcoat color change from pale greenish yellow (PGY) to SGY. Through crosses with genetic tester stocks, the seedcoat genotype of 'Enola' was determined to be $C\ J\ g\ b\ v^{lae}\ Rk$. The test cross 'Enola' x 'Wagenaar' demonstrated that 'Enola' also carries the gy gene. The relationship of 'Enola' to the 'Mayocoba' market class of common bean and to

'Azufrado Peruano 87' is discussed.

Prakken (1970) summarized the genetics of seedcoat colors in common bean (*Phaseolus vulgaris*) other than red colors, and he reconciled the various systems of gene symbols used by various researchers. Subsequently, Prakken (1972) published his extensive work with red seedcoat colors and organized the entire body of seedcoat color genetics (Prakken, 1970, 1972) into two tables, one for the yellow-black series of colors and the other (a text table) for the red colors. One of the colors in the first table is pale greenish yellow (canary), which was also called schamois by Lamprecht (1932). The genotype of pale greenish yellow is *P C J g b v Rk*. The seedcoat color investigated in the present paper is a very much more intense color, which we will designate as strong greenish yellow (SGY).

The seedcoat genotype of 'Wagenaar' was studied by Prakken (1940) in the cross 'Wagenaar' x 'Citroen' and found to be $P C D J g b v^{lae}$ (using the gene symbols of Prakken, 1970). In subsequent work, Prakken (1972) used 'Wagenaar' in two diallel crossing sets of four parents each, and he analyzed the 12 resulting F_2 progenies. This work confirmed the previous genotype for seedcoat color, which is described as "shiny pale greenish yellow" with purple corona. The purple corona trait is controlled by the v^{lae} gene (Bassett, 1995a).

Prakken (1940) described 'Wagenaar' as a two-toned seedcoat (canary and schamois), which he interpreted as being always typical of the seedcoat genotype $P C J g b v$ (or v^{lae}). The senior author of this paper has never observed expression of the canary yellow seedcoat color in the genetic stock $g b v$ BC$_3$ 5-593, which he describes as (very) pale greenish yellow (PGY). In this paper the canary color of Prakken (1940) will be called SGY.

When 'Wagenaar' is grown in the greenhouse at Gainesville, Fla., or Fargo, N. Dak., the distribution of SGY on the seedcoats of 'Wagenaar' is often incomplete, i.e., part of the seedcoat has PGY and the remainder SGY. Our observations for two-toned color pattern are the same as those of Prakken (1940), but our genetic interpretation is different. We hypothesize that the genotype $C J g b v$ gives schamois to (very) pale greenish yellow, but never the SGY (canary) that Prakken (1940) observed in 'Wagenaar.' Furthermore, we hypothesize that an independent gene (tentative symbol *Gy*) with variable expressivity intensifies PGY to SGY in 'Wagenaar.' The same SGY color of 'Wagenaar' occurs with variable expressivity in the 'Mayocoba' market class of dry (common) bean (Bassett, unpublished observation). 'Enola', a patented dry bean cultivar, also has the SGY color with variable expressivity as shown in the color photograph included in the patent (Proctor, 1999). Therefore, the objectives of this work were to 1) determine the inheritance of SGY seedcoat color, 2) reexamine the full seedcoat genotype of 'Wagenaar', 3) test alternative hypotheses that propose that SGY is produced either by a mutant allele at the *G* (yellow seed color) locus or by a gene independent of *G*, using a combination of classical and molecular genetic approaches, 4) determine the seedcoat genotype of 'Enola' and the 'Mayocoba' market class, and 5) discuss the claims of the 'Enola' patent in relation to the findings of this paper.

Materials and Methods

Eight genes control seedcoat color in common bean, and very complicated epistatic interactions occur among those genes

[1]Professor.

[2]Technician.

[3]Undergraduate student.

(Prakken, 1972). For this paper, only a brief introduction to the genetics of seedcoat color is needed. The cultivars tested in this paper all carried the dominant (wild type) allele at the P, C, J, and Rk loci, and those dominant alleles do not alter the color. Similarly, the cultivars tested all carried the recessive r allele at the R locus for dominant red color, which is closely linked to C. The genes G, B, and V are color modifying genes: G (from *Gelbe*, a German word) for yellow with $G\ b\ v$, B for mineral brown with $G\ B\ v$, and V for violet to black (anthocyanin pigments) with $G\ B\ V$. With $g\ b\ v$, the seedcoat is nearly colorless, shamois to (very) pale greenish yellow or cream color. This paper presents evidence for a ninth seedcoat color gene expressing SGY in the $P\ [C\ r]\ J\ g\ b\ v\ Rk$ genetic background.

Seeds of 'Wagenaar' were obtained from H. Dijkstra, Collection Manager, Centre for Genetic Resources, Wageningen, The Netherlands. Prakken (1940) described a difference in the color of 'Wagenaar' "between the hilum side and the opposite dorsal (and lateral) side of the seed" as being characteristic. The hilum (ventral) side was canary yellow, whereas the dorsal side was schamois. The two color zones were not sharply separated, and transitional colors occurred. Prakken (1940) goes on to comment, "The canary yellow is extremely variable in its extension, even in seeds on the same plant; sometimes it is nearly imperceptible or restricted to very small ventral spots in the region of the germ root and near the caruncula; in other cases nearly the whole seedcoat can show the color." This exact and detailed description fits perfectly the observed seedcoat color of 'Wagenaar' when grown at Gainesville, Fla., or Fargo, N. Dak.

Seeds of the 'Mayocoba' market class were obtained from the Los Angeles market by a source that cannot be disclosed due to contractual agreement. Seeds of 'Enola' were obtained from Mark Brick, Colorado State University, Fort Collins. Our dry bean breeding line 5-593 (Florida) has small seed size, with shiny black seedcoats of genotype $T\ P\ [C\ r]\ D\ J\ G\ B\ V\ Rk$ (Bassett, 1994, 1996a; Bassett and Blom, 1991; Prakken, 1970). Genetic tester stocks were developed by using 5-593 as the recurrent parent in backcrossing programs (with F_2 selection in each cycle) to transfer recessive genes, singly and in combination, into this standard genetic background. The genetic tester stocks used as testcross parents with 'Wagenaar' and 'Enola' are listed and described in Table 1.

Development of the genetic tester stock $g\ b\ v$ BC$_3$ 5-593 began with a cross of 'Calima' ($[C^{ma}\ R]\ J\ g\ b\ v\ Rk$) with $G\ b\ v$ BC$_2$ 5-593 (Bassett, unpublished data). From this cross, a true breeding F_3 progeny was developed with PGY seedcoat color ($[C\ r]\ J\ g\ b\ v$).

This F_3 was crossed with $G\,b\,v\,BC_3$ 5-593 to create $g\,b\,v\,BC_1$ 5-593. In a similar manner, two additional backcrosses (with F_2 selection in each cycle) to $G\,b\,v\,BC_3$ 5-593 were used to create $g\,b\,v\,BC_3$ 5-593 in Spring 1997.

Over the past several years, the genetic tester stocks P_3, P_5, P_6, P_8, and P_9 (Table 1) were all crossed with 'Wagenaar', and the F_1 progeny of the test crosses were grown in the greenhouse at Gainesville, Fla., to produce the most complete expression of the seedcoat genes involved. Data were recorded for flower color and seedcoat color and pattern of the F_2 seed produced. During the greenhouse season of 1999-2000, 'Enola' was crossed with genetic tester stocks P_1, P_3, P_4, P_7, and P_9 (Table 1), and the F_1 progeny of the test crosses were grown in the greenhouse at Gainesville, Fla. The F_2 progeny of the 'Enola' test crosses were grown in the field in Spring 2000. Flower color and seedcoat color and pattern phenotypes of the parental, F_1, F_2, and F_3 seed were recorded. Plants of 'Mayocoba' were grown in the greenhouse along side 'Enola' plants in 1999. Plant type and seed color data were recorded and used to further compare 'Mayocoba' and 'Enola'.

Test crosses for allelism of the SGY seedcoat color were made between 'Wagenaar' x 'Mayocoba' and 'Enola' x 'Wagenaar', and the F_1 progeny were grown in the greenhouse at Gainesville, Fla. Data were recorded on seedcoat color. From the cross 'Enola' x 'Wagenaar', seeds were harvested from 10 F_2 plants (bulked seed) selected for full SGY seedcoat color development. The SGY seedcoat color was characterized using two methods: 1) the Munsell Book of Color (1966 edition, 2.5R-10G, Munsell Color Co., Inc., Baltimore, Md.) and 2) a chromameter (model CR-200; Minolta, Ramsey, N. J.). For the latter technique, a seed sample was placed on a black table top as background. Data were recorded for both methods of characterization of SGY color.

The cross 'Wagenaar' x $g\,b\,v\,BC_3$ 5-593 was made and 80 F_2 plants were grown in the greenhouse at Fargo, N. Dak. All seeds from each F_2 plant were harvested. The plants were also classified for flower color and seedcoat pattern. A complete genetic model for the phenotypic data was developed, and the genetic segregation data were analyzed using the orthogonal contrasts of Mather (1957). Genetic linkage was calculated by the maximum likelihood method and tables of Allard (1956).

All seeds from each F_2 plant from the cross 'Wagenaar' x $g\,b\,v\,BC_3$ 5-593 were planted in the field at Gainesville, Fla., in Spring 2000 to achieve an F_3 progeny analysis of the F_2. Due to variable expressivity of the SGY trait, the seed sampling procedure varied depending on the phenotype of the F_2 parent. Prog

Table 1. Seedcoat phenotypes and genotypes of 'Wagenaar' and 'Enola' and the genetic stocks used in testcrosses to determine the seedcoat genotype of 'Wagenaar' and 'Enola' common bean.

Parent Stock		Seedcoat color		
no.	name	Phenotype	Genotype	Reference
P_1	Wagenaar	Strong greenish yellow with purple corona	$P\,C\,D\,J\,g\,b$ v^{lae}	Prakken, 1972

P_2	Enola	Strong greenish yellow	Unknown	
P_3	c^u BC$_3$ 5-593	Cartridge buff	$P\,c^u\,D\,J\,G\,B\,V$	Bassett, 1996a
P_4	$c^u\,b\,v\,rk^d$ BC$_1$ 5-593	Dark red kidney	$P\,c^u\,D\,J\,G\,b\,v\,rk^d$	Bassett, unpublished
P_5	$[c\,R]\,b\,v$ BC$_3$ 5-593	Oxblood red	$P\,[c\,R]\,D\,J\,G\,b\,v$	Bassett, 1996b
P_6	j BC$_3$ 5-593	Dull dark purple with margo pattern[z]	$P\,C\,D\,j\,G\,B\,V$	Bassett, 1996a
P_7	dj BC$_3$ 5-593	Dull dark purple with white hilum ring and corona	$P\,C\,dj\,G\,B\,V$	Bassett, 1996a
P_8	$G\,b\,v$ BC$_3$ 5-593	Yellow brown	$P\,C\,D\,J\,G\,b\,v$	Bassett, 1995b
P_9	$g\,b\,v$ BC$_3$ 5-593	Pale greenish yellow	$P\,C\,D\,J\,g\,b\,v$	

[z]Margo pattern has colored hilum ring, white (or nearly white) corona, and greater loss of dark purple color on the dorsal side of the seed than on the ventral side.

Table 2. Results of testcrosses between 'Wagenaar' common bean and a series of genetic stocks with known seedcoat genotypes.

Testcross[z]	Phenotype of seedcoats of seeds on F_1 plants from the testcross
P_1 x P_3	Black/cartridge buff marbled
P_1 x P_5	Red/yellow brown (with red haze) mottle (subtle). At first glance the seed looks all red like the tester. A low contrast mottle pattern not typical for the $C/[c\,R]$ interaction, i.e., this is a true C/c mottle.
P_1 x P_6	Shiny black seed (no margo pattern)
P_1 x P_8	Violet (yellow brown with reddish haze)/pale yellow brown mottle (very subtle); purple corona
P_1 x P_9	Pale violet/pale greenish yellow mottle; purple corona

[z]The names, phenotypes, and genotypes of the parental lines (P_i) are given in Table 1.

enies from PGY F_2 parents had only one pod sampled and a single seed selected for the composite for that plot. Progenies from SGY F_2 parents had all pods harvested, and the entire seed production of each plant was classified for seedcoat color by scoring all seeds individually. F_3 plants that produced any seeds with SGY color were classified as SGY. There were 76 F_3 progenies grown, including a total of 670 plants. The mean F_3 consisted of 8.8 plants, with the range of 1 to 27 plants.

Following the procedures of Brady et al. (1998), randomly amplified polymorphic DNA (RAPD) markers were developed for two seedcoat color genes: G and a putative new gene

controlling SGY color and tentatively given the symbol *Gy*. The *G* locus markers were developed from the F_2 from the cross *g b v* BC_2 5-593 x *G b v* BC_3 5-593 (P_8). The genetic stock *g b v* BC_2 5-593 is an earliler backcross version of P_9 (Table 1). A RAPD marker for the *Gy* locus was developed from the F_2 population of the 'Wagenaar' x *g b v* BC_3 5-593 cross described above.

Results and Discussion

CHARACTERIZATION OF SGY SEEDCOAT COLOR. The observed color descriptors of the color tiles in the Munsell Book of Color most closely resembling the bulked seed sample from selected F_2 plants from the cross 'Enola' x 'Wagenaar' ranged from 5Y 8/6 to 8/10 and 7/8 to 7/10, and7.5Y 8/6 to 8/8 and 7/10. Those values describe the stronger greenish yellow of our selected seed materials compared with the paler greenish yellow of the seed materials described in the 'Enola' patent (Proctor, 1999). Using the same seed sample with the Minolta Chroma Meter, the objective color values recorded were L = 52.87, C = 36.13, and H = 86.9.

A NEW LOCUS FOR SGY VS. A MUTANT *G* GENE. Before this research, two competing hypotheses existed regarding the SGY seedcoat color phenotype. One hypothesis suggested the novel seedcoat color was the result of another allele at the classic *G* gene. Alternatively, it was hypothesized that the SGY seedcoat color phenotype was the result of a new gene that modified the PGY seedcoat color phenotype. Our first approach to testing the alternative hypotheses was to develop molecular markers linked to *G* and determine if they were linked to or cosegregated with the SGY phenotype.

An F_2 population (derived from the cross *g b v* BC_2 5-593 x *G b v* BC_3 5-593) segregating at *G* was used to identify a RAPD linked to the gene. The population segregated for yellow-brown (*G_*) and PGY (*gg*) seedcoat color in the expected 3:1 phenotypic ratio ($\chi^2_{3:1}$ = 1.67, *P* = 0.19) for this dominant-acting gene. Two bulks were created, each consisting of a pool of eight DNA samples from the yellow-brown or PGY seedcoat color classes. The DNA bulks were amplified by polymerase chain reaction (PCR) using a series of primers, and three primers were discovered that produced amplification patterns in which a fragment was present in the yellow-brown (*G_*) but not the PGY (*gg*) seedcoat color bulks. DNA from each member of the segregating population was amplified with the three primers, and the fragment segregation patterns were identical. Each individual in the population that contained the fragment was the *G_* genotype, and each individual lacking the fragment was the *gg* genotype. Recombination was not observed; and, therefore, the three marker fragments, $OAP7_{850}$, $OAP3_{1400}$, and $OU14_{950}$, appear to cosegregate with *G*. In addition, the three markers cosegregated with respect to each other.

The next experiment tested the linkage between one *G*-linked marker ($OAP7_{850}$) and the SGY seedcoat color phenotype. An F_2 population from the cross 'Wagenaar' x *g b v* BC_3 5-593 (P_1 x P_9 of Table 2) segregated 3:1 for the PGY and SGY phenotypes (Table 3). Each individual in this population was scored for the presence and absence of the $OAP7_{850}$ marker. This *G* marker and the two seedcoat color phenotypes segregated independently in this population. We, therefore, hypothesized that the genetic factor controlling the SGY seedcoat color was not *G*.

The next step was to develop a molecular marker linked to a gene for the SGY seedcoat color phenotype. A bulk segregant procedure, similar to that described above for the G marker development, was used to discover marker $OAP12_{1400}$ that is linked in coupling with the dominant PGY phenotype. As described below, F_2 individuals were genotypically classified using F_3 family analysis. Using this data, the linkage between Gy and

Table 3. Segregation for seedcoat color in F_2 and F_3 from the cross 'Wagenaar' x $g\ b\ v$ BC_3 5-593 (pale greenish yellow tester).

F_2 segregation			F_3 segregation				
No. of plants[z]	Seedcoat color	Genetic hypothesis	No. of progenies[y]	PGY[x] $Gy/-$	SGY[w] gy/gy	χ^2 3:1	P
69	PGY[x]	$Gy/-$	34	All			
			28	251	69	2.017	0.16
14	SGY[w]	gy/gy	14	All			

[z]For the F_2 segregation data 69 and 14, the $\chi^2_{3:1}$ = 2.928, P = 0.09.

[y]For the F_2 segregation data 34, 28, and 14, the $\chi^2_{1:2:1}$ = 15.79, $P < 0.001$.

[x]PGY = pale greenish yellow seedcoat.

[w]SGY = strong greenish yellow seedcoat.

Table. 4. Segregation for seedcoat color and pattern in the F_2 from the cross 'Wagenaar' ($C.\ J\ g\ b\ v^{lae}$ gy) x $C\ J\ g\ b\ v\ Gy$ BC_3 5-593.

No. of plants[z]	Phenotype[y] Seedcoat	Genotype		
20	Violet, purple corona	$Gy/-$	C/C	$v^{lae}/-$
27	Violet/PGY mottled, purple corona	$Gy/-$	C/C^r	$v^{lae}/-$
13	Violet, PGY corona	$Gy/-$	C/C	v/v
4	Violet/PGY mottled, PGY corona	$Gy/-$	C/C^r	v/v
7	SGY, purple corona	gy/gy	$-/-$	$v^{lae}/-$
9	SGY, PGY corona	gy/gy	$-/-$	v/v

[z]Combining classes without regard for the C locus, the data 47, 17, 7, 9 give $\chi^2_{9:3:3:1}$ = 7.822, P = 0.05. The orthogonal contrasts are χ^2_{Gy} = 1.067, P = 0.30, χ^2_{V} = 2.400, P = 0.12, and χ^2_{L} = 4.356, P = 0.037; coupling linkage between Gy and V is 35.51 ± 6.938 cM.

[y]PGY = pale greenish yellow, SGY = strong greenish yellow.

the $OAP12_{1400}$ marker was determined to be 7.5 cM. To further test the independence of G and the gene controlling the SGY phenotype, the G segregating population was scored with the $OAP12_{1400}$ marker. The marker and the G locus segregated independently. These

molecular genetic tests lead us to the conclusion that the SGY phenotype derived from 'Wagenaar' is controlled by a genetic factor other than the G locus. At this point, the inheritance of this trait was studied in more detail.

'WAGENAAR' TEST CROSSES. 'Wagenaar' was crossed to a series of genetic tester stocks with known genotypes. The F_1 phenotypes (F_2 seed) are described below (Table 2). The cross with P_3 produced a marbled seedcoat with black and cartridge buff. The genetic interpretation is that 'Wagenaar' carries C (See, Bassett, 2000, Table 3, class 5). The interpretation of the test cross with P_3 is limited to determining that 'Wagenaar' does not carry c^u or the classic c allele of Lamprecht (1932). The 'Wagenaar' C gene, nevertheless, carries a linked mottling function, as will be presented and discussed below. The cross with P_5 produced a surprising result (Table 2). The expected yellow brown color had a red haze, for which no hypothesis is pursued in this paper. The genetic interpretation is that 'Wagenaar' carries r. The test cross with P_6 gave a nonallelic interaction, indicating that 'Wagenaar' carries J (Table 2). The test crosses with P_8 and P_9 gave allelic interactions at G and B, but not for the V locus. The presence of purple corona color indicates that 'Wagenaar' carries v^{lae}. Thus, the genotype of 'Wagenaar' has been confirmed to be $C J g b v^{lae}$, which is in agreement of the findings of Prakken (1972). Only the finding of the red haze over yellow brown in the test cross with P_5 is a new result. Prakken (1972) also established that 'Wagenaar' carries the gene Rk at the red kidney locus, and our results are consistent with that genotype.

The F_1 from the cross 'Wagenaar' x $g b v$ BC$_3$ 5-593 (P_1 x P_9 of Table 2) produced the PGY (in light pattern color areas of the seedcoat) of P_9. This result supports the hypothesis that the SGY of P_1 is a recessive trait. The segregation for seedcoat color in the F_2 from the cross P_1 x P_9 fit a 3:1 ratio for PGY seedcoats to SGY seedcoats, respectively (Table 3). The data were consistent with the hypothesis that SGY is controlled by a single recessive gene.

F3 TEST OF Gy HYPOTHESIS AND VARIABLE EXPRESSIVITY. The hypothesis of a single recessive-acting gene for SGY was tested in F_3. The 14 F_2 parents with SGY seedcoat color were true breeding in F_3 progenies (Table 3). The 28 F_2 parents with PGY seedcoats that segregated for PGY and SGY seedcoats in F_3 did so in a 3:1 ratio, respectively (Table 3). The remaining 34 F_2 parents with PGY seedcoats were true breeding. The above three classes of F_3 progenies failed to fit the expected 1:2:1 ratio for true breeding SGY, segregating progenies, and true breeding PGY progenies (Table 3). The failure was due to an excess of true breeding PGY progenies. Low F_2 seed yield probably prevented the accurate F_3 characterization of some PGY F_2 individuals as heterozygotes. The hypothesis that the SGY trait is controlled by a single recessive-acting gene is supported by two results from the F_3 test: 1) the SGY class was true breeding and 2) a 3:1 segregation ratio for PGY and SGY, respectively, observed for the heterozygous F_3 families.

We propose the gene symbol gy for the SGY trait. Although current rules for gene symbol

nomenclature in common bean usually require three letters for the gene symbol, an exception was made to avoid making strings of gene symbols for seedcoat color genotypes any longer than necessary. The Genetics Committee of the Bean Improvement Cooperative has approved the gene symbol gy for SGY trait. We avoided using the gene symbol sgy for good reason. Although seeds with genotype $C J g b v$ have shamois color, in this paper we retained the name pale greenish yellow (PGY) in deference to the summary table of Prakken (1972). In a future revision of this table the color name for genotype $C J g b v$ should be changed to shamois only. Apparently, Lamprecht never reported observing a seed stock with gy (Bassett, personal review of all Lamprecht papers), and Prakken never did genetic analysis with a seed stock having $C J g b v Gy$. Besides 'Wagenaar', the only other stocks with $g b v^{lae}$ used by Prakken (1972) had C^m or C^{st} (both with R expressed in the dark pattern areas).

The SGY character showed variable expressivity in the F_3

Table 5. Results of testcrosses between 'Enola' common bean and a series of genetic stocks with known genotypes.

Phenotypes of flowers and seedcoats on F_1 plants from the testcross

Testcross[z]	Flower[y]	Seedcoat
$P_2 \times P_7$	C.V.	Shiny black seed (no margo pattern)
$P_2 \times P_3$	C.V	Dark mineral brown/cartridge buff marbling
$P_2 \times P_4$	P.P.	Yellow brown/cartridge buff marbling; no purple corona
$P_2 \times P_9$	P.P.	Violet/pale greenish yellow marbling; no purple corona

[z] The names, phenotypes, and genotypes of the parental lines (P_i) are given in Table 1.

[y] C.V. = cobalt violet color expressed by V/v^{lae}, P.P. = pale pink expressed by v^{lae}/v.

progenies derived from SGY F_2 parents. There was great variation from seed to seed within plants, from plant to plant within plots, and between plots for the frequency of extensively SGY colored seedcoats (data not presented). Although the data were not sufficient to develop a genetic model for the inheritance of higher and more stable expression of the SGY trait, the data suggested that other genetic factors may control higher expression levels.

For the cross 'Wagenaar' x $g b v$ BC$_3$ 5-593, the F_1 progeny had mottled pale violet/PGY seedcoats (Table 2), and the F_2 progeny segregated for the same phenotype (Table 4). This mottled phenotype was not expressed well in the F_3 progeny grown in the field, and no data were recorded for mottling in that generation. Although 'Wagenaar' has the dominant C gene (Table 2) (Bassett, 2000; Prakken, 1972), the mottling function (property) of the 'Wagenaar' C is designated by the symbol C' (Table 4). Interestingly, the mottling effect from C' does not express with gy/gy, and the SGY trait does not express (giving PGY by default) in the corona region with gy/gy v/v (Table 4). Thus, with gy/gy there is no seedcoat expression for the distinction between the C/C and C/C' genotypes (Table 4). Two possible interpretations are that Gy may be 1) linked to C or 2) be an allele at C. Using the BAT 93 x

Jalo restriction fragment length polymorphism (RFLP) mapping system (Nodari et al., 1993), the sequence tagged site (STS) marker developed from the RAPD marker OAP12$_{1400}$ was mapped to linkage group B8, showing two map units between the C and Gy loci (McClean, personal communication). Thus, the data support close linkage, but not allelism at C. Similar procedures with the same mapping system demonstrated that V is located in linkage group B6. Hence, the weak linkage between Gy and V of about 35 cM (Table 4) was found to be artifactual (McClean, personal communication).

'ENOLA' TEST CROSSES AND RELATIONSHIP TO 'MAYOCOBA'. The cross P_2 x P_3 gave F_2 seeds with dark mineral brown/cartidge buff marbling, which is interpreted as a C gene in 'Enola' (Bassett, 2000) although a black/cartridge buff marbling is expected (Table 5). The cross P_2 x P_7 gave F_2 seeds with black color without pattern (a nonallelic response to the j in the tester), which is interpreted as a J gene in 'Enola' (Table 5). The cross P_2 x P_4 gave F_2 seeds with yellow brown/cartridge buff marbling with no purple corona, which is an allelic response for b and a nonalleleic response for rk^d and rk (Table 5). Surprisingly, the cross P_2 x P_9 gave F_2 seeds with violet/pale greenish yellow marbling and no purple corona, which is an allelic response for g, b, and v (Table 5). No hypothesis for the violet color will be pursued in this paper. The pink flower color of 'Enola' indicates that 'Enola' carries v^{lae} (Prakken, 1970). The cross P_2 x P_1 gave F_2 seeds with SGY color, which is an allelic response indicating that 'Enola' also carries gy. Thus, the seedcoat genotype $C J g b v^{lae} Rk gy$ for 'Enola' has been demonstrated.

'Enola' has pink flowers that are known to result from expression of the gene v^{lae}, but the seedcoat does not have the purple corona color produced pleiotropically by v^{lae} (Bassett, 1995a; Prakken, 1970). Both the corona and hilum ring of 'Enola' are either SGY or PGY, whereas the hilum ring color produced by $C J g b v$ is brown and by $C J g b v^{lae}$ is dark purple (Prakken, 1970). The test cross 'Enola' x 'Wagenaar' produced F_2 seeds with SGY corona and hilum ring (data not presented). Similarly, the testcrosses P_2 x P_4 and P_2 x P_9 failed to show the expected purple corona, but, on the other hand, the flower color phenotypes for the four test crosses with 'Enola' support the hypothesis that v^{lae} is present in 'Enola' (Table 5). Our hypothesis is that 'Enola' carries an unknown, dominant epistatic gene that suppresses the expected dark purple corona and brown hilum ring.

Both 'Enola' and 'Mayocoba' have the same SGY seedcoat color as 'Wagenaar,' and both cultivars express the same SGY color in the corona and hilum ring in the presence of gene v^{lae}. The test cross 'Wagenaar' x 'Mayocoba' produced F_2 seeds with SGY color, purple corona, and brown hilum ring (data not presented). Thus, although 'Enola' and 'Mayocoba' both carry the gy gene for SGY, our hypothesis is that 'Mayocoba' carries an unknown, recessive epistatic gene that suppresses the expected dark corona and brown hilum ring in 'Mayocoba'. A full investigation of the interaction of v^{lae} (and the C and J genes for hilum ring color) with both dominant and recessive epistatic suppressor genes is beyond the scope of this paper.

Comparison of the plant structure of 'Enola' and the 'Mayocoba' stock used in this paper showed that they were virtually indistinguishable (data not presented). These appearance similarities are consistent with the hypothesis that 'Enola' is a selection from one of the

pure-line commercial cultivars of the 'Mayocoba' market class grown in Mexico for export to the United States market. In the 1960's, or by early 1970 at the latest, the land race Canario (same as U.S. 'Mayocoba' class) was sent from Peru to Mexico (O. Voysest, personal communication). This was the first time that 'Mayocoba' beans were introduced into Mexico. The Mexicans crossed 'Canario Divex 8120' x 'Canario 107' and released the derivative cultivar 'Azufrado Pimono 78' in 1978. This began a new commercial class in Mexico, which they designate "Peruano" (Voysest, 2000). In the early 1980's the name of 'Azufrado Pimono 78' was changed to 'Mayocoba'. This very popular Mexican cultivar name was, thereafter, used to denote the market class in the United States. More than five other Peruano cultivars were developed in Mexico after 'Mayocoba'. In 1987, Mexican bean breeder Ingeniero Salinas and colleagues released 'Azufrado Peruano 87', a Peruano class bean cultivar (Kelly, 2000). After the 'Enola' patent (Proctor, 1999) was issued, a biotechnology laboratory in Texacoco, Mexico, determined by DNA analysis of 'Enola' (seeds obtained from the U.S. Patent Office) that 'Enola' was derived from 'Azufrado Peruano 87' (Kelly, 2000).

The patent for 'Enola' (Proctor, 1999) claims that "Enola seeds possess a unique yellow color...", but the results given above demonstrate that the well-known 'Wagenaar' bean cultivar, as well as all the Peruano market class cultivars of Mexico, have the same seedcoat color. When 'Enola' and 'Mayocoba' are grown together in the same greenhouse in Gainesville, the SGY seedcoat color of both cultivars is strongly expressed and covers the entire seedcoat of nearly all seeds when plants mature in December; but when the same cultivars mature together in March, the seedcoat color is distributed incompletely on the seedcoat and is weaker in expression. This seasonal variation is also typical of 'Wagenaar' when grown in greenhouse culture and is typical for a trait with varible expressivity. The 'Enola' patent (Proctor, 1999) also claims that the yellow color of the seed remains uniform and stable from season to season., but our results do not support that claim. The 'Enola' patent (Proctor, 1999) makes an exclusive property claim to all bean cultivars with the seedcoat color of 'Enola' (referred to as SGY in this paper) based on "invention" of that seedcoat color, but we assert that the program (described in the patent) of several successive cycles of self-pollination and selection from yellow bean materials purchased in Mexico did not create or invent the seedcoat color of 'Enola', i.e., the "invention" has no basis in fact.

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